REVIEW ARTICLE

Na^+/Ca^{2+} exchangers: three mammalian gene families control Ca^{2+} transport

Jonathan LYTTON[1]

Department of Biochemistry and Molecular Biology, Libin Cardiovascular Institute of Alberta, Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Mammalian Na^+/Ca^{2+} exchangers are members of three branches of a much larger family of transport proteins [the CaCA (Ca^{2+}/cation antiporter) superfamily] whose main role is to provide control of Ca^{2+} flux across the plasma membranes or intracellular compartments. Since cytosolic levels of Ca^{2+} are much lower than those found extracellularly or in sequestered stores, the major function of Na^+/Ca^{2+} exchangers is to extrude Ca^{2+} from the cytoplasm. The exchangers are, however, fully reversible and thus, under special conditions of subcellular localization and compartmentalized ion gradients, Na^+/Ca^{2+} exchangers may allow Ca^{2+} entry and may play more specialized roles in Ca^{2+} movement between compartments. The NCX (Na^+/Ca^{2+} exchanger) [SLC (solute carrier) 8] branch of Na^+/Ca^{2+} exchangers comprises three members: NCX1 has been most extensively studied, and is broadly expressed with particular abundance in heart, brain and kidney, NCX2 is expressed in brain, and NCX3 is expressed in brain and skeletal muscle. The NCX proteins subserve a variety of roles, depending upon the site of expression. These include cardiac excitation–contraction coupling, neuronal signalling and Ca^{2+} reabsorption in the kidney. The NCKX (Na^{2+}/Ca^{2+}–K^+ exchanger) (SLC24) branch of Na^+/Ca^{2+} exchangers transport K^+ and Ca^{2+} in exchange for Na^+, and comprises five members: NCKX1 is expressed in retinal rod photoreceptors, NCKX2 is expressed in cone photoreceptors and in neurons throughout the brain, NCKX3 and NCKX4 are abundant in brain, but have a broader tissue distribution, and NCKX5 is expressed in skin, retinal epithelium and brain. The NCKX proteins probably play a particularly prominent role in regulating Ca^{2+} flux in environments which experience wide and frequent fluctuations in Na^+ concentration. Until recently, the range of functions that NCKX proteins play was generally underappreciated. This situation is now changing rapidly as evidence emerges for roles including photoreceptor adaptation, synaptic plasticity and skin pigmentation. The CCX (Ca^{2+}/cation exchanger) branch has only one mammalian member, NCKX6 or NCLX (Na^+/Ca^{2+}–Li^+ exchanger), whose physiological function remains unclear, despite a broad pattern of expression.

Key words: calcium transport, excitation–contraction coupling, melanosome biogenesis, renal calcium reabsorption, signal transduction, sodium/calcium exchanger, synaptic plasticity.

INTRODUCTION

Ionized calcium (Ca^{2+}) is utilized by cells as a ubiquitous signalling molecule, and changes in its concentration control a variety of diverse physiological events from the beginning of life to its end, as well as at many points in between. It is now widely appreciated that specificity in Ca^{2+} signalling is achieved largely through complex spatial and temporal dynamics brought about by the integrated action of a large set of channels and transporters that move Ca^{2+} across membranes between different compartments. Alteration in the function or regulation of any of these carefully choreographed players has serious deleterious consequences, often associated with the pathogenesis of disease [1,2].

Na^+/Ca^{2+} EXCHANGERS: FROM DISCOVERY TO THE MOLECULAR ERA

Among the different pathways that mediate Ca^{2+} movement, Na^+/Ca^{2+} exchange has emerged as the predominant mechanism for Ca^{2+} efflux across the plasma membrane, particularly when overall Ca^{2+} levels are high [3–5]. Moreover, several recent studies have highlighted the connection between important physiological events and specific Na^+/Ca^{2+} exchanger molecules [6–8]. Na^+/Ca^{2+} exchange was first described in squid axon and mammalian heart some four decades ago. Extensive studies demonstrated that Na^+/Ca^{2+} exchange played a crucial role in Ca^{2+} extrusion, and operated with a stoichiometry of three Na^+ ions to one Ca^{2+} ion, indicating that the transport process was electrogenic [9]. The development of partially purified exchanger preparations, antibody reagents and expression cloning techniques led to the molecular cloning of the canine cardiac Na^+/Ca^{2+} exchanger, subsequently denoted NCX1 [10]. The *NCX1* cDNA encoded a protein of 970 amino acids predicted to be approx. 110 kDa in size. The mature protein is subject to both signal peptide cleavage and glycosylation, and, when analysed on gels, runs with an apparent size of 120 kDa. Additional bands are observed at 160 and 70 kDa, which arise by heat-induced aggregation and proteolytic cleavage respectively. Further minor variations in apparent size (5–10 kDa) are generated by alternative splicing.

Parallel studies in retinal rod photoreceptors had established the presence there of a mechanistically similar Na^+/Ca^{2+}-exchange process that is critical for visual adaptation and represents the predominant means of Ca^{2+} extrusion from rod outer segments [11]. The rod exchanger differed from the one described in heart and axon principally due to its absolute transport requirement for K^+ ions. These studies indicated that the rod exchanger catalysed the transport of 4 Na^+ in exchange for 1 Ca^{2+} and 1 K^+ [12,13]. Purification of the bovine rod Na^+/Ca^{2+}–K^+ exchanger

Abbreviations used: CaCA, Ca^{2+}/cation antiporter; CAX, Ca^{2+}/anion exchanger; CBD, Ca^{2+}-binding domain; CCX, Ca^{2+}/cation exchanger; DREAM, downstream regulatory element antagonist modulator; HEK-293, human embryonic kidney; LTP, long-term potentiation; NCKX, Na^+/Ca^{2+}–K^+ exchanger; NCLX, Na^+/Ca^{2+}–Li^+ exchanger; NCX, Na^+/Ca^{2+} exchanger; PIP_2, phosphatidylinositol bisphosphate; PKA, protein kinase A; PKC, protein kinase C; PLM, phospholemman; SLC, solute carrier; SR, sarcoplasmic reticulum; XIP, exchanger inhibitory peptide.

[1] email jlytton@ucalgary.ca

subsequently led to its molecular cloning [14,15]. This cDNA, denoted *NCKX1*, encoded a protein of 1216 amino acids predicted to be approx. 132 kDa in size. Extensive glycosylation of the protein appears to account for the much larger apparent size of the native protein observed on gels ($\geqslant$ 220 kDa). Despite their obvious mechanistic and architectural similarities, NCX1 and NCKX1 share only very limited sequence identity, within a duplicated motif referred to as the α-repeat (see below).

A SUPERFAMILY OF Na^+/Ca^{2+} EXCHANGERS

These initial molecular cloning events led to a rapid explosion of sequence information on related molecules that were isolated by low-stringency library screening or database mining. Soon it was established that *NCX1* was part of larger gene family, SLC (solute carrier) 8, whereas *NCKX1* was the founding member of another family, SLC24. More recent analysis has established that SLC8 and SLC24 are both part of a larger superfamily of related Ca^{2+}/cation antiporter genes, the CaCA superfamily [16–18] (see Figure 1). This superfamily has five major branches, tentatively named after characterized representative members: (i) YRBG, comprising largely bacterial members; (ii) CAX (Ca^{2+}/anion exchanger), comprising mostly plant and yeast members; (iii) NCX (SLC8) and (iv) NCKX (SLC24), both comprising almost exclusively vertebrate members; and (v) CCX (Ca^{2+}/cation exchanger), which contains the partially characterized molecule previously called NCKX6 or NCLX (Na^+/Ca^{2+}–Li^+ exchanger). The YRBG and CAX branches of the family probably catalyse H^+/Ca^{2+} exchange; NCX and NCKX, of course, catalyse Na^+/Ca^{2+} exchange, and the CCX family has been demonstrated to catalyse both Na^+/Ca^{2+} exchange and Li^+/Ca^{2+} exchange, but, by virtue of its phylogeny in a separate branch, may have an as yet undetermined endogenous specificity. Mammals express three members of the NCX (SLC8) family, *NCX1*, *NCX2* and *NCX3* [10,19,20] (a new fourth member of the NCX family has recently been identified in teleost fish [21], but is not present in mammalian genomes); five members of the NCKX (SLC24) family: *NCKX1*, *NCKX2*, *NCKX3*, *NCKX4* and *NCKX5* [15,22–25]; whereas *NCKX6*/*NCLX* is the lone member of the CCX branch found in mammals [26,27].

The CaCA superfamily of transporters is characterized by a conserved overall membrane topology comprising two clusters of five hydrophobic putative transmembrane helices, joined by a cytoplasmic loop of varying length. Certain members of the family appear to possess, in addition, a cleaved putative signal peptide at the N-terminus and extra (or sometimes fewer) putative transmembrane spans in each hydrophobic cluster [17] (Figure 1). In addition to this characteristic membrane topology, the CaCA superfamily is also defined by the presence of a signature conserved amino acid sequence motif present within each hydrophobic cluster, the so-called α-repeat regions [16]. Residues within these regions have been demonstrated through mutagenesis experiments to be critical for the transport function of both NCX1 [28–31] and NCKX2 [32,33], and are thought to form the ion-binding pocket of these exchangers through the physical association of two α-repeats, one from each hydrophobic cluster, oriented in opposite directions [29,34–39]. The two α-repeat regions together with the repeated pattern of hydrophobicity, indicate that these molecules each comprise a duplicated structure which presumably has its origins in a dimeric dual-topology membrane protein [40,41], although none has been identified thus far that is related specifically to the CaCA superfamily. The remainder of this review will focus on members of the CaCA superfamily that are expressed in mammals.

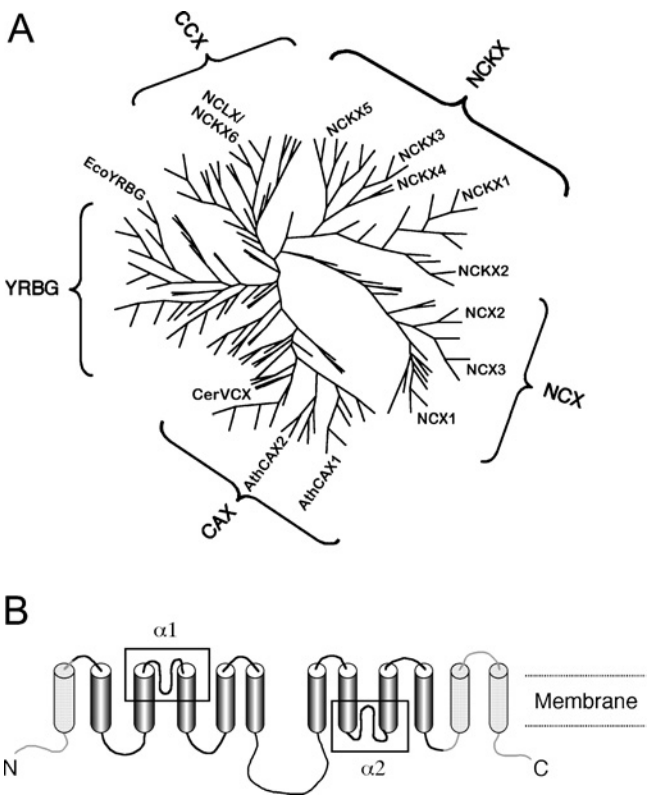

Figure 1 CaCA phylogeny and Na^+/Ca^{2+} exchangers

(**A**) Phylogenetic tree of 147 unique members of the CaCA superfamily is shown. Five major branches in the tree are labelled, and representative members are shown (GenBank® accession numbers are given in parentheses): EcoYRBG, the YRBG protein from *Escherichia coli* (NP_417663); CerVCX, the vacuolar Ca^{2+}/H^+ exchanger from *Saccharomyces cerevisiae*, (NP_010155); AthCAX1 and AthCAX2, Ca^{2+}/H^+ exchangers from *Arabidopsis thaliana* (NP_181352 and NP_566452); NCX1, NCX2 and NCX3, Na^+/Ca^{2+} exchangers from *Homo sapiens* (NP_066920, NP_055878 and NP_150287); NCKX1, NCKX2, NCKX3, NCKX4 and NCKX5, Na^+/Ca^{2+}–K^+ exchangers from *Homo sapiens* (NP_004718, NP_065077, NP_065740, NP_705932 and NP_995322); NCLX/NCKX6 from *Homo sapiens* (NP_079235). (**B**) Putative general topology model for proteins of the CaCA superfamily. Hydrophobic presumed transmembrane helical segments are illustrated as cylinders and are linked to show the topological connectivity. The conserved α-repeat regions are shown boxed and oppositely oriented with respect to the membrane. The N-terminal transmembrane segment (light grey) is absent from some, and is thought to be removed by a co- or post-synthetic proteolytic cleavage event in other, members. The C-terminal two-transmembrane spans (light grey) are variably present in different family members. Additionally, and not shown, the CCX branch is predicted to have an additional hairpin pair of transmembrane helices between the central cytosolic loop and the α-2 motif. Modified from [17] with permission from Oxford University Press.

THE SLC8 Na^+/Ca^{2+} EXCHANGER FAMILY

NCX1 is the most broadly expressed member of the SLC8 family. Originally characterized and cloned from heart, it was subsequently realized that *NCX1* is also highly expressed in brain and kidney, and at lower levels in almost all other tissues [42–44]. Transcripts of the *NCX1* gene are alternatively spliced at two sites in a tissue-specific manner [43,45]. The first site lies in the 5′-untranslated region and does not alter the structure of the encoded protein. Instead, the presence of different non-coding initiating exons is a consequence of three independent promoters selectively driving and regulating expression of NCX1 in different tissues: one specific to heart, one to kidney, and one for other tissues [46,47]. Presumably this situation provides the capacity to independently regulate gene expression in response to tissue-specific physiological demands.

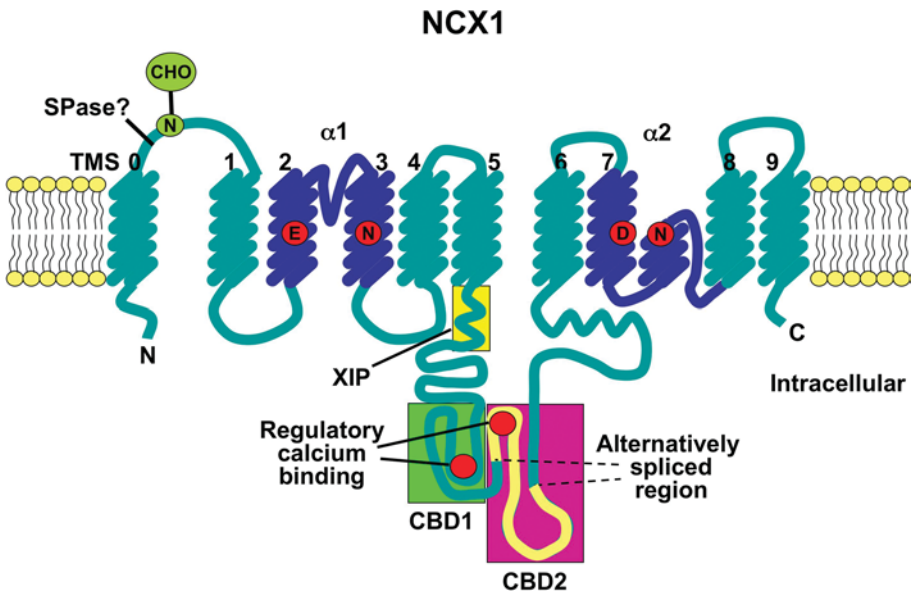

Figure 2 Topology model for NCX1

The experimentally supported model for NCX1 has nine proposed transmembrane-spanning segments (TMSs), a cleaved 'signal peptide' (TMS0, with the putative signal peptide cleavage site shown as SPase?) and re-entrant loops, one of which may be partially helical, as part of the conserved α-repeat motifs (dark blue, labelled α1 and α2). Critical amino acids in the α-repeat regions are highlighted in red (Glu^{113}, Asn^{143}, Asp^{814} and Asn^{842}). The single N-linked glycosylation site is shown in green (N-CHO). Highlighted regions of the cytosolic loop include the XIP, the two CBDs (CBD1 and CBD2) and the region subject to alternative splicing. Proposed sites involved in regulatory Ca^{2+} binding are also shown.

The second site of alternative splicing lies within the coding region of the transcript, where two mutually exclusive and four cassette exons encode a potentially large number of protein isoforms with variations in a cytosolic segment of the protein [43,45,48]. The mutually exclusive exons appear to be incorporated such that transcripts in excitable tissues and cells (primarily muscle and nerve) include one exon (exon A), whereas transcripts in non-excitable cells and tissues include the other (exon B). No obvious tissue-specific pattern has been observed for the cassette exons. In contrast with *NCX1*, the other members of the SLC8 family have a much more restricted pattern of expression. *NCX2* is abundant in neurons in all parts of the brain, but not present at significant levels elsewhere. *NCX3* is expressed selectively in skeletal muscle and at lower levels in some brain regions [49]. *NCX3*, but probably not *NCX2*, is alternatively spliced to generate variability in a similar cytoplasmic region as *NCX1*.

The three mammalian SLC8 family members display a high degree of sequence identity throughout the length of the protein. Thus, whereas almost all of the structural and functional work has been performed on NCX1, the conclusions are also likely to apply, at least in general terms, to NCX2 and NCX3 [44]. Aside from recent studies on part of the cytoplasmic loop (see below), information regarding the structure of NCX1 has largely been inferred indirectly from biochemical or mutagenic analyses. The overall architecture of the NCX proteins is well established and supported by experimental evidence (see Figure 2). The N-terminus acts as a 'signal peptide' and is cleaved co- or post-translationally [50–53], and thus the N-terminus of the mature protein is extracellular and part of a glycosylated region [54], which is followed by a hydrophobic region containing the first of the two α-repeats, then by a large cytoplasmic loop, and finally a second hydrophobic region containing the second α-repeat. The protein C-terminus is thought to be on the intracellular side of the membrane [34,55]. The predicted size of the NCX2 and NCX3 proteins are 100 and 103 kDa respectively, which corresponds closely to their observed size on gels [56].

THE NCX1 MEMBRANE DOMAIN

The precise topological folding arrangement of the polypeptide within the two hydrophobic membrane-embedded regions has been studied extensively. The current model proposes five transmembrane helices in the first hydrophobic cluster and four in the second for NCX1. The conserved α-repeats of NCX1 contain amino acids that are essential for function, including two critical acidic residues, one within each α-repeat [28–30]. Accessibility experiments indicate that the two α-repeats are oriented in opposite directions with respect to the membrane, with their central regions probably forming membrane re-entrant loops that have limited accessibility to both sides of the membrane [29,34]. Cross-linking experiments have localized the helices predicted to flank the α-repeats in proximity with one another [35,36]. Thus it seems most probable that the two α-repeats form part of a vestibule or pathway required for the binding and transport of ions through the NCX1 protein.

Not all the cross-linking data are compatible with the simple all-helical model for the two hydrophobic clusters, however [35,36]. Instead, these data suggest that the α-repeats may form a more convoluted, intramembrane, structure which would be consistent with the presence of a number of helix-breaking residues present in these motifs. There is thus still much to be learned about the nature of the ion-binding sites in Na^+/Ca^{2+} exchangers, including how one site can accommodate either one Ca^{2+} or three Na^+ ions in a mutually exclusive manner, and why reorientation of the ion-binding site accessibility from one side of the membrane to the other requires its occupancy. It is possible that cation binding at the two key acidic amino acids is an essential step required to allow reorientation and transport. This model would be similar

in principle to that proposed for the *Escherichia coli* GlpT transporter [57].

THE NCX1 CYTOPLASMIC DOMAIN

The cytoplasmic region is responsible for complex and physiologically important modes of NCX1 regulation, as well as being the target for several recently identified inhibitory compounds. Gaining structural insight into this critical region of NCX1 will thus provide a new opportunity to develop a mechanistic understanding of regulation and inhibition. Linearly, the central cytoplasmic loop of NCX1 can be broken down into different motifs (Figure 2). Beginning at the N-terminal end, adjacent to the first cluster of membrane-spanning helices, is an amphipathic sequence, called the 'XIP' (exchanger inhibitory peptide) region because exogenous addition of a peptide with this sequence inhibits its exchanger function [10,58]. This region has been implicated in regulation of NCX1 function by both Na^+ and by acidic phospholipids (see below). The XIP region is followed by a sequence modelled to be similar to a catenin-like domain, then by two so-called β-repeats, originally identified as similar to β4 integrin [16], and more recently the subject of two structural studies [59,60]. It is now clear that each β-repeat together with flanking sequence corresponds to a CBD (Ca^{2+}-binding domain) with an immunoglobulin-like fold comprising a seven-stranded β-sandwich structure (see Figure 3).

The first of these domains (CBD1), binds four Ca^{2+} ions with high affinity at one end of the β-sandwich [60], an event associated with a conformational change that is thought to be responsible for the known regulatory effect of Ca^{2+} binding on NCX1 activity (see below). The second domain (CBD2) forms a structure homologous with CBD1 that binds Ca^{2+} with lower affinity and without a corresponding large conformational change [59]. CBD2 also contains the region of variability owing to alternative splicing. The two CBDs are thought to pack together in an antiparallel manner with the Ca^{2+}-binding end of CBD1 pointing into the cytoplasm away from the membrane [59]. The mutually exclusive alternatively spliced exons form a central and essential structural role in CBD2. However, the variable cassette exon portion of the alternatively spliced region resides in a loop of CBD2 that faces away from the membrane (see Figure 3). The C-terminal end of the cytoplasmic domain is composed of a hydrophobic and proline-rich sequence that was originally modelled as a transmembrane span. Subsequent experimental evidence indicates that this sequence actually lies on the cytoplasmic side of the membrane [34]. Despite its unusual sequence and somewhat conserved nature among other NC(K)X family members, no function for this region has yet emerged. The cytoplasmic loops of NCX2 and NCX3 display a relatively high degree of amino acid identity with that of NCX1, which indicates that the overall domain structure is likely to be conserved. Consistent with this view, both NCX2 and NCX3 display regulatory behaviour similar to that of NCX1 [61].

NCX1 TRANSPORT FUNCTION

Operation of the Na^+/Ca^{2+} exchanger is fully reversible, and so the direction of ion movement depends entirely upon the electrochemical ion gradients and the number of ions that bind and are transported. For NCX1, the stoichiometry of ion transport has been investigated extensively for many years using a variety of techniques and a value of three Na^+ ions in exchange for one Ca^{2+} ion has generally been accepted [9]. The resulting unequal movement of charge means that NCX1 transport is electrogenic [62], and thus can be measured using electrophysiology [63].

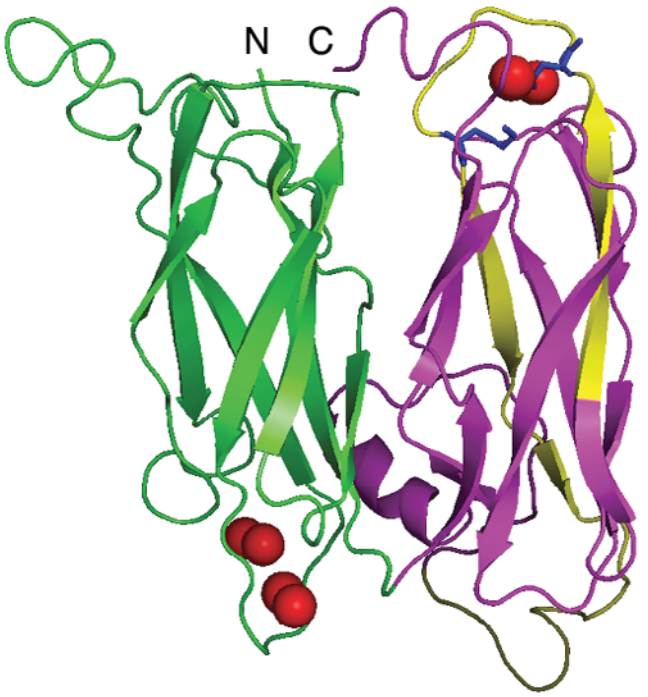

Figure 3 Structural model for the NCX1 CBDs

Cartoon representations of the two CBD structures [59,60] (CBD1 is green, and CBD2 is magenta, with the region subject to alternative splicing, Thr^{570}–Ala^{609}, in yellow) are shown juxtaposed as they may pack in the native protein. The membrane plane lies at the top, orthogonal to the orientation of the Figure, connected to the CBDs via their labelled N- and C-terminal ends. The orientation is similar to that represented in Figure 2. Red spheres indicate the location of bound Ca^{2+} ions, and the two key amino acids that define the difference in properties between the mutually exclusive alternatively spliced exons A and B are shown as stick representations in blue (Asp^{578} and Lys^{585}). Figure prepared with the PDB files 2FWS, 2FWU and 2DPK using PyMOL software (http://www.pymol.org). The 2FWS and 2FWU structures, as well as the location of the four Ca^{2+} ions in CBD1 from 2DPK, were used to prepare a model illustrating a possible packing arrangement, according to [59]. Note that, although based on structural data, this Figure represents a possible composite model and not an actual structure. An interactive three-dimensional version of this Figure is available at http://www.BiochemJ.org/bj/406/0365/bj4060365add1.htm.

Several studies using ionic current to measure activity suggested a Na^+/Ca^{2+} stoichiometry of 4:1 [64,65]. Although a rare (1 Ca^{2+}+1 Na^+) transport mode has been proposed to explain these apparent discrepancies in stoichiometry [66], it is not clear that the stoichiometry issue has been adequately resolved. Na^+/Ca^{2+} exchange is thought to be mediated by a sequential series of binding, conformational changes and unbinding steps that involve one ion-binding site that can accommodate either three Na^+ ions or one Ca^{2+} ion [except as proposed above for the rare (1 Ca^{2+}+1 Na^+) transport mode] [9]. Full occupancy of the site allows transport of the bound ion(s) across the membrane. This sequential mechanism, and the requirement for occupancy of the ion-binding site with either Na^+ or Ca^{2+}, accounts for the observation that the exchanger can catalyse Na^+/Ca^{2+} exchange, as well as Na^+/Na^+ exchange and Ca^{2+}/Ca^{2+} exchange [67] (see Figure 4).

Under normal resting cellular ionic conditions and membrane potential, NCX1 operating with a 3:1 stoichiometry will act to extrude Ca^{2+} from the cytoplasm [9]. In the heart, during systole when the membrane becomes depolarized and Na^+ levels rise adjacent to the plasma membrane by flow through voltage-operated Na^+ channels, NCX1 reverses operation and allows a small amount of Ca^{2+} entry [68]. This Ca^{2+}, while not directly triggering SR (sarcoplasmic reticulum) release and inducing contraction, may play an important regulatory role in the

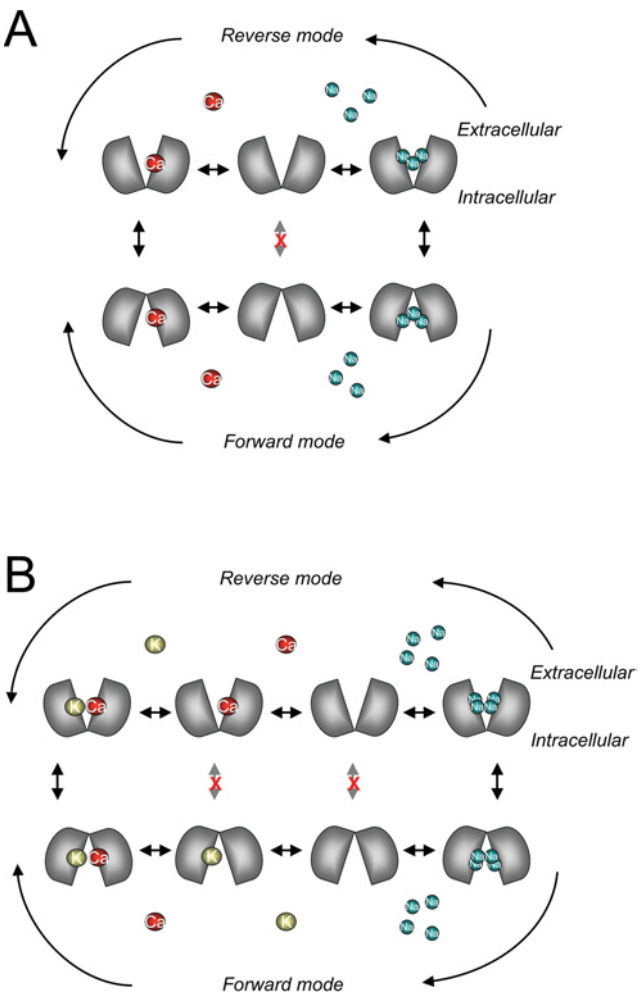

Figure 4 Proposed transport cycle for Na^+/Ca^{2+} exchangers

(**A**) The NCX1 cycle. The empty exchanger is shown to bind either one Ca^{2+} ion or three Na^+ ions, in a mutually exclusive manner. Either binding event allows a conformational change that reorients the accessibility of the ion-binding site across the membrane barrier. Forward-mode Ca^{2+} extrusion in exchange for Na^+ entry occurs by sequential steps in a clockwise direction. Conversely, reverse-mode Ca^{2+} influx in exchange for Na^+ exit proceeds around the cycle in an anti-clockwise direction. (**B**) The NCKX cycle. As in (**A**), except the empty transporter binds either one Ca^{2+} ion plus one K^+ ion or four Na^+ ions. The binding of Ca^{2+} and K^+ is shown ordered based on unpublished work (F. Visser and J. Lytton) and because kinetic considerations under normal physiological ion concentrations suggest that the cycle is likely to be limited by Ca^{2+} binding to an exchanger with a cytoplasmically oriented site primed with bound K^+. An animated version of this Figure is available at http://www.BiochemJ.org/bj/406/0365/bj4060365add2.htm.

excitation–contraction coupling process by influencing the gating of neighbouring voltage-operated Ca^{2+} channels and by altering the SR Ca^{2+} load. The functional interconnectivity of these different ion channels and transporters in the cardiac sarcolemma and SR is facilitated by the apposition of the two membrane systems, which creates a restricted diffusional, or 'fuzzy', space where ion concentrations can reach much higher levels than detected globally [69]. The importance of this physical arrangement in the context of excitation–contraction coupling is evident from the large physical protein complexes that normally exist here [70,71], and the serious consequences to heart function when they cannot form because of the absence of the scaffolding protein ankyrin [72]. The stoichiometry of ion transport has not been established experimentally for NCX2 or NCX3. On the basis of the high level of sequence identity within the putative transport regions, however, it is anticipated that these two proteins will behave identically with NCX1.

REGULATION OF NCX1 FUNCTION

Although the direction of Ca^{2+} flux through NCX1 depends upon the thermodynamic factors discussed above, the rate of transport depends upon kinetic factors, influenced by the amount of exchange protein present, the occupancy of the ion-binding sites and regulation of activity. Expression of the *NCX1* gene in heart displays developmental regulation, being higher in neonatal animals and then declining gradually with age [73–76]. Such an expression pattern is consistent with the timing of SR and SR–T-tubule junction development, and the switch of cardiomyocyte Ca^{2+} handling from the plasma membrane to the SR [77]. NCX1 expression is also increased in cultured cardiomyocytes following α-adrenergic stimulation [46]. Otherwise, most studies examining transcriptional regulation of the NCX1 gene have focused on changes associated with pathological scenarios such as cardiac overload or end-stage heart disease, where expression of the molecule is generally up-regulated [78–81].

Regulation of the NCX2 and NCX3 genes has been studied in cell culture systems. When cerebellar granule cells are grown in the presence of high potassium concentrations, conditions which promote their differentiation, the expression of NCX2 is down-regulated via a Ca^{2+}/calcineurin-dependent mechanism, while expression of NCX3 is up-regulated [82]. Further investigation of the regulation of NCX3 has revealed an important role for the Ca^{2+}-binding transcriptional regulator, DREAM (downstream regulatory element antagonist modulator). In this model, elevated [Ca^{2+}] binds DREAM, which relieves its action as a repressor, resulting in gene activation [83]. These observations suggest intriguing Ca^{2+}-dependent mechanisms for regulation of Ca^{2+} transporters and may explain expression of specific gene products in environments that experience large fluctuations in [Ca^{2+}].

The intracellular ion transport site of NCX1 has an apparent affinity for Ca^{2+} in the low micromolar range, and so the exchanger is not maximally activated until [Ca^{2+}] rises well above resting levels. This site also has an apparent affinity for Na^+ of approx. 10–20 mM which is close to or a little higher than normal resting [Na^+]. The externally facing transport site has markedly different apparent affinities for Ca^{2+} and Na^+ ions of approx. 1 and 60–80 mM respectively [63,84,85]. Thus the overall kinetic rate of the exchanger under normal resting cellular conditions is limited principally by occupancy of the cytoplasmic Ca^{2+}-binding sites. In addition to regulation by substrate levels, a number of other factors have been found to regulate NCX1 activity allosterically. These include Ca^{2+}, Na^+, PIP_2 (phosphatidylinositol bisphosphate) and other acidic phospholipids, H^+ and a variety of interacting 'partner' proteins [86].

Regulation of NCX1 activity by Ca^{2+} was first observed using dialysed squid axon, and then in patch-clamped myocytes and giant excised patches [63,87,88]. Measurement of the exchanger in the reverse, Ca^{2+}-entry, mode revealed the essential requirement of Ca^{2+} at the cytoplasmic surface for activity. Although as measured experimentally, Ca^{2+} activation of NCX1 is relatively slow [89,90], at least in principle this form of regulation might take place in a cardiac cell on a beat-to-beat basis [91]. Treatment of the excised patches with chymotrypsin removes Ca^{2+} regulation, leading to constitutive activation of the exchanger [89]. Acidic clusters within the central cytosolic loop of the exchanger were shown later to be critical for Ca^{2+} regulation [92,93] and, as presented above, most recently the CBDs have been characterized structurally [59,60]. Although evidence for conformational changes of these domains in response to Ca^{2+} binding has been

measured both biophysically and biochemically, a mechanistic model to explain regulation of exchanger activity is still lacking. Curiously, the *Drosophila melanogaster* orthologue of NCX1 (CALX1) is inhibited, rather than activated, by Ca^{2+} binding to a similar region [94]. Chimaeric constructs revealed that the stimulatory property of Ca^{2+} binding could be re-introduced partially into CALX1 together with a region encompassing CBD1 from mammalian NCX1 [95].

While cytosolic Ca^{2+} activates the exchanger, high levels of cytosolic [Na^+] have been shown to inhibit activity [89,96]. This so-called Na^+-dependent inactivation is observed as a significant decline to a steady-state level following the rapidly developing current that declines with time to a steady-state level is measured when Na^+ is applied to activate NCX1 transport in excised patches. Na^+-dependent inactivation was originally modelled to take place following Na^+ binding to the cytoplasmically oriented transport site, via a transition to an inactive conformation of the exchanger [96]. An alternative model has also been proposed, in which Na^+ interacts with a regulatory site distinct from the transport site [86]. Mutations in the amphipathic XIP region at the N-terminus of the cytosolic domain have been shown to have a major influence on Na^+-dependent inactivation, some mutations preventing inactivation, and some enhancing it [97].

ATP was recognized to be an important activator of NCX1 when measured using the giant excised patch preparation [89,98]. The ATP effect is predominantly as a consequence of preventing Na^+-dependent inactivation, and appears to involve the production of the phosphorylated phospholipid, PIP_2 [99]. Further investigation of the mechanism revealed that the XIP region was involved in PIP_2 activation, as might be expected based on the interaction of this domain with Na^+-dependent inactivation [100]. Key mutations in the XIP region have reciprocal effects on Na^+-dependent inactivation and PIP_2 activation. NCX1 has also been demonstrated to be regulated by a variety of other lipids [101–104]. A recent intriguing report suggested that NCX1 in heart may be regulated in a manner co-ordinated with fat metabolism via activation by long-chain acyl-CoA esters [105]. This activation was shown to involve the XIP region, in a manner analogous to activation by PIP_2, although the mechanism appeared to be different.

Na^+-dependent inactivation of NCX1 can also be prevented by raising pH [106], or by increasing Ca^{2+} to levels higher than those required for Ca^{2+} activation [98]. The alleviation of inactivation by Ca^{2+} is only observed in those NCX1 isoforms containing the mutually exclusive alternatively spliced exon A (i.e. NCX1.1, NCX1.4 and NCX1.5) [107]. Mutagenesis studies revealed two key amino acids within exon A that are required for this effect, Asp^{578} and Lys^{585}, which are part of the Ca^{2+}-binding loop of CBD2 [108] (see Figure 3). It seems plausible that these two residues are required to maintain a conformation of CBD2 that is competent to bind Ca^{2+}. Whether the pH effect on Na^+-dependent inactivation occurs via CBD2, the XIP region, or elsewhere in the NCX1 molecule, remains unclear.

It would seem that regulation of NCX1 function by Na^+, Ca^{2+} and PIP_2 as described above is in general poised to enhance activity of the exchanger in promoting Ca^{2+} extrusion and reducing Ca^{2+} entry modes of operation. Furthermore, these regulatory influences seem too slow to regulate the exchanger significantly during the time course of one heart beat, but are more likely to respond integratively to longer-term homoeostatic influences [109]. Thus, for example, ischaemic conditions that result in elevated intracellular Na^+ and reduced ATP levels would be expected to shift NCX1 into the reverse mode of operation both thermodynamically and kinetically. These conditions, however, lead to regulatory inactivation of the exchanger, hence minimizing Ca^{2+} entry. In contrast, raising cytosolic Ca^{2+} will activate the forward operation of NCX1 thermodynamically, kinetically and via the dual regulatory influences of Ca^{2+} activation and the relief of Na^+ inactivation. Thus a developing theme is the functional and mechanistic interconnectivity between the different regulators of NCX1 activity: Ca^{2+}, Na^+, PIP_2 and pH. The recent determination of the CBD structures [59,60] provides an opportunity to advance our understanding of the mechanism underlying these related modes of NCX1 regulation to the molecular level.

The interaction of NCX1 with partner proteins has emerged recently as another critical modulatory influence on function. That the exchanger might interact with other proteins was first suggested following studies that demonstrated a specific localization of NCX1 to T-tubule membranes in cardiomyocytes [110], to plasma membrane domains overlying SR elements in smooth muscle cells [111], and on the basolateral membrane of renal epithelial cells [112]. An association between NCX1 and the cytoskeletal protein ankyrin was subsequently observed *in vitro* [113]. More recently, analysis of cardiomyocytes from ankyrin B-knockout heterozygous mice revealed decreased stability, reduced expression and mislocalization of NCX1 [114,115]. Presumably this arises from disorganization of the cytoskeletal network owing to reduced levels of ankyrin expression. As ankyrin is capable of binding to multiple proteins and linking them to the cytoskeleton, ankyrin interactions may underlie the observations that NCX1 is part of a macromolecular complex [70,71]. Such a complex may help co-ordinate Ca^{2+} signalling in cardiac myocytes and other cells. The site of ankyrin binding to NCX1 has not yet been identified, but one might anticipate that it would be found in the large central cytosolic loop of the protein. Importantly, an interaction within this region has the potential to have significant influences on NCX1 function and its regulation above and beyond localization.

An interaction of NCX1 with the other cytoskeletal proteins has also been investigated. In particular, there is evidence that an interaction with the filamentous actin network influences both cell-surface delivery of NCX1, as well as playing an important role in modulating regulation via the central cytoplasmic loop [116,117]. Whether these effects of actin involve a direct protein interaction, or a more indirect one via a larger macromolecular complex, remain unknown.

Caveolins are small lipophilic scaffolding proteins identified as forming a coat structure on the cytoplasmic side of plasma membrane invaginations known as caveolae [118,119]. A variety of Ca^{2+}-sensitive signalling molecules cluster together in caveolae, possibly leading to integrated actions involved in Ca^{2+} homoeostasis [120,121]. Caveolae are enriched in cholesterol and sphingolipids, and hence these structures, and the caveolin proteins associated with them, can also be isolated biochemically as ‘lipid rafts’. The caveolin-3 isoform is expressed in heart, preferentially present on the surface membrane of ventricular myocytes, where it appears to play a key role in the regulation of normal cardiac function [121,122]. Although NCX1 is mostly present in the T-tubule membrane, a significant fraction of these two molecules overlap [123], and caveolin-3 and NCX1 have been shown in some studies to co-associate [124,125]. Other studies, however, do not support this association [126]. The reason for these different results is unclear, but might reflect different conditions or animal models. In addition to the situation in heart, NCX1 in neuronal cells has been shown to associate, at least partially, with caveolin-1 and -2 [127].

The number of protein partners shown to interact with NCX1 has increased dramatically in the last few years. PLM (phospholemman) is one of the better characterized of these [128]. PLM is

a member of the so-called 'FXYD' family of transport regulators, best know as modulators of Na^+, K^+-ATPase activity in various tissues [129]. When phosphorylated at Ser^{68} by PKA (protein kinase A), the C-terminal cytosolic tail of PLM binds to two regions of the NCX1 cytosolic loop, inhibiting NCX1 function [130–132]. This action would be parsimonious with the overall influence of β-adrenergic agonists to promote Ca^{2+} entry, SR loading and the contractility of cardiomyocytes. Consistent with these findings, PLM-null mice exhibit enhanced NCX1 function [133]. PLM has also been demonstrated to have an activating influence on cardiac Na^+,K^+-ATPase [134]. As NCX1 and the Na^+,K^+-ATPase are thought to co-associate in a macromolecular complex within the T-tubule [70], co-ordinate regulation of these functionally linked transporters may well be of physiological importance.

Two more recently discovered NCX1-interacting partners, both identified using yeast two-hybrid screening strategies, were calcineurin [135] and the 14-3-3 proteins [136]. In both instances, the interactions were inhibitory. Binding of the Ca^{2+}-sensitive phosphatase, calcineurin, to the cytosolic loop of NCX1 was enhanced in phenylephrine-induced hypertrophy, and may be responsible, at least in part, for the observed decrease in NCX1 activity observed under these conditions [135]. The 14-3-3 proteins are a large family of proteins implicated in a variety of signalling cascades as a result of their property of promiscuous binding to phosphorylated serine and threonine residues in target proteins [137]. Several different 14-3-3 protein family members are able to bind to the cytosolic loops of NCX1, NCX2 and NCX3 in a phosphorylation-dependent manner, inducing inhibition of activity [136]. The physiological or pathophysiological consequences of 14-3-3 binding have yet to be defined.

Phosphorylation of NCX1 remains a topic of much controversy. Several studies have suggested that NCX1 function can be modulated by adrenergic stimulation, operating via PKA and/or PKC (protein kinase C), in both myocytes and recombinant systems [61,138–141]. Furthermore, there is evidence for direct phosphorylation of the NCX1 protein, when expressed heterologously [71,141]. However, further studies have revealed that, at least in certain cases, adrenergic activation of Na^+/Ca^{2+} exchange can be explained either by indirect effects on local substrate concentration or by effects on contaminating activities not related to the exchanger [142,143]. It remains possible that another contributing factor to this controversy is the different sensitivity of NCX1 in different animal species (particularly frogs) [144]. In addition, although activation of PKC has been shown in heterologous systems to result in both phosphorylation of NCX1 and stimulation of exchange activity [145], subsequent experiments demonstrated that the phosphorylation events were not related to changes in activity [146]. It remains possible, however, that NCX1 activity is regulated indirectly, via its participation in macromolecular complexes with other proteins, including PLM [70,71,128]. Extensive experiments in squid axon, where phosphorylation of a small regulatory protein stimulates exchanger activity, are supportive of such a model [86].

NCX1 AND PHYSIOLOGY

The role of the Na^+/Ca^{2+} exchanger in normal cellular physiology has in the past either been inferred from theoretical considerations and from examining its function in isolation under artificial conditions or has been determined by employing crude ion substitution experiments or by using inhibitors of doubtful selectivity (such as benzamil) [9]. Although these approaches have allowed the broadly based conclusion that a major role for the exchanger is in the removal of Ca^{2+} from cells, the nature of the invasive approaches has precluded detailed study, particularly under physiological conditions. More recently, the generation of more selective inhibitors and, most importantly, genetic approaches, has allowed new fascinating insights [6,147].

In the heart, contraction is initiated by a small influx of Ca^{2+} across the plasma membrane that then induces a larger release from the SR. The relative magnitude of these two components differs from species to species, ranging from less than 10 % influx in rodents to approx. 30 % influx in humans [68]. Relaxation requires Ca^{2+} removal and sequestration in proportion with prior influx and release, in order to maintain long-term cellular Ca^{2+} homoeostasis. Measured activity levels of Na^+/Ca^{2+} exchange compared with plasma membrane Ca^{2+}-ATPase clearly indicated that the exchanger was the predominant Ca^{2+} efflux pathway and therefore essential to cardiac Ca^{2+} homoeostasis and hence function [9]. Ca^{2+} extrusion via the exchanger also has the secondary consequence of generating a depolarizing current (carried by Na^+ ions) which may contribute to the shape and length of the action potential. In addition, theoretical considerations suggest that at the peak of the action potential, thermodynamic conditions favour Ca^{2+} entry via NCX1. The extent to which this Ca^{2+} entry contributes to excitation–contraction coupling in myocytes has been controversial, since, among other things, the precise concentration of ions under the plasma membrane experienced by the exchanger is uncertain [68]. These uncertainties speak of the difficulties in distinguishing NCX1 function under physiological conditions.

The critical nature of NCX1 activity to cardiac function was originally supported by studies in mice where global knockout of the *NCX1* gene had an embryonically lethal phenotype at approx. 8.5 days post-conception, which was attributed to a cardiac defect [148–151]. More recent studies, however, suggest that the cause of lethality in *NCX1*-knockout mice may be extra-cardiac in origin [152]. Still, loss of NCX1 function during development cannot be compensated for by other members of the NCX family or by other Ca^{2+} transport mechanisms. Surprisingly, cardiac-specific knockout of *NCX1* was not lethal [153]. Only a modest deficit in cardiac function was present in young animals, although this did progress to the development of cardiac hypertrophy and ultimately heart failure as the animals aged.

Myocytes from these knockout animals display remarkably normal Ca^{2+} dynamics. More detailed examination revealed that the knockout myocytes have a dramatically reduced L-type Ca^{2+} channel current (although there was no loss of $Ca_v1.2$ protein), that could be normalized upon dialysis with the Ca^{2+}-chelator BAPTA [bis-(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetra-acetic acid] [154]. This result suggests that reduced Ca^{2+} clearance from the diadic cleft in the absence of NCX1 leads to enhanced Ca^{2+}-dependent inactivation of the L-type Ca^{2+} channel in the knockout myocytes. Additionally, the myocytes had a dramatically shortened action potential due to up-regulation in the hyperpolarizing I_{Ko} (outward K^+ current), and an increase in expression of K^+ channel subunits $K_v4.2$ and KChIP (K^+ channel-interacting protein) [155]. The combined effect of a much shorter action potential and a rapidly inactivating Ca^{2+} current was that knockout myocytes had an 80 % reduction in the flux of Ca^{2+} across the sarcolemma. The fact that this dramatic reduction in trigger Ca^{2+} did not result in reduced SR Ca^{2+} release and reduced contractility was due to an increase in the gain of excitation–contraction coupling. The mechanism that underlies this change in gain is currently not known. Ultimately, however, operating at maximal gain prevents changes in Ca^{2+} homoeostasis needed to respond to increases in demand for cardiac output. Consequently, this dysregulation of

integrated Ca^{2+} transport leads in the long term to hypertrophy and heart failure.

Interestingly, examination of transgenic mice that overexpress NCX1 demonstrate precisely the converse alterations in sarcolemmal properties. Namely, an increase in the L-type Ca^{2+} current and an elongation of the action potential plateau phase [156]. This fascinating and remarkable series of compensatory changes clearly illustrates the finely tuned and highly interdependent network of protein molecules responsible for overall Ca^{2+} homoeostasis in heart cells. Presumably, changes in local [Ca^{2+}] itself, or possibly alterations in multimeric protein complexes associated with the NCX1 protein, underlie the adaptations to large shifts in NCX1 protein expression in these genetically altered myocytes.

The extent to which the exchanger can reverse during the action potential to deliver trigger Ca^{2+} for further SR release has been a subject of considerable controversy [68]. New data from cardiac-specific *NCX1*-knockout mice suggest that normally reverse exchange activity at the peak of the action potential helps to load the diadic cleft, and hence synchronize rapid SR release [157]. In the absence of NCX1, Ca^{2+} does not build up as quickly. Instead, the lack of NCX1-driven Ca^{2+} removal leads to a slower and larger build up of diadic Ca^{2+}, and hence a slight delay in SR Ca^{2+} release.

The role of the exchanger in other tissues has not been examined in the same detail as it has in the heart [9]. In brain, crude ion substitution experiments as well as the high density of expression of the Na^+/Ca^{2+} exchanger localized to sites of high plasmalemmal Ca^{2+} flux, such as presynaptic terminals, have all led to the main conclusion that the role of the exchanger in neurons is to extrude Ca^{2+} following its entry, hence terminating signalling events [49,158]. The role of the exchanger in brain neurons is complicated further by the expression of at least two NCX subtypes here, NCX1 and NCX2, as well as lower and more selective expression of NCX3 [49,159]. The widespread high level of NCX2 expression implies a key role in neuronal Ca^{2+} homoeostasis and function (see below). There is also evidence that NCX3 plays a selective and critical role in protecting cerebellar granule cells from ischaemic insults [56,160]. Further details regarding the localization of NCX1, NCX2 and NCX3 expression in different neurons, as well as the precise subcellular distribution of the three proteins, will provide insight into possible distinct physiological roles for each of these different gene products [161].

The Ca^{2+} extrusion mode of the Na^+/Ca^{2+} exchanger generates an inward, depolarizing, current as a consequence of the uneven charge translocation during turnover. When coupled to the extrusion of Ca^{2+} due to a signal initiated by entry across the plasma membrane, this depolarization can have a role in shaping an action potential, or can even induce a feedback effect on the entry pathway (reducing the driving force for Ca^{2+} entry). The inward Na^+/Ca^{2+} exchanger current due to efflux of Ca^{2+} released from intracellular stores can be an important component of downstream signalling, by modulating other voltage-gated channels. For example, serotonin and orexin, acting via their receptors to release Ca^{2+} from intracellular stores, are thought to depolarize their target neurons, at least in part, via this mechanism [162–164].

As discussed above, relatively subtle alterations in either membrane potential or ion concentration close to the membrane can induce the Na^+/Ca^{2+} exchanger to reverse operation and catalyse the influx of Ca^{2+} instead of its extrusion. This mode of operation is thought to contribute significantly to Ca^{2+} influx induced by, among other signals, the neurotransmitter glutamate, presumably as a consequence of Na^+ entry through ionotropic glutamate receptors [165,166]. These actions of the Na^+/Ca^{2+} exchanger are unlikely to play a major role during the course of a single action potential, but are more likely to be important in slower processes or longer-term cumulative effects. Repetitive rapid action potential trains, as found in some neurons, could lead to persistent elevation in Na^+, which via its influence on Na^+/Ca^{2+} exchanger function, would induce a longer-term modulation of [Ca^{2+}]. This complexity of roles for a Na^+/Ca^{2+} exchanger in brain neurons presumably reflects the molecular diversity of structures encoding Na^+/Ca^{2+} exchanger molecules, as well as the formation of protein complexes and functional coupling mediated by local ion gradients in restricted diffusional spaces.

The *NCX1* gene product is relatively abundant in a variety of smooth muscles [167]. Indeed, altering Na^+/Ca^{2+} exchanger activity can have a large effect on subsequent agonist-induced contraction [168–170]. However, few studies have been able to demonstrate a reliable correlation between inhibition of Na^+/Ca^{2+} exchanger function and changes in cytosolic Ca^{2+}. Subcellular localization of NCX1 in arterial smooth muscle cells revealed that the protein was present in domains of the plasma membrane associated with underlying SR elements [111]. The Na^+/Ca^{2+} exchanger therefore has a privileged indirect communication with the SR stores, operating through a highly restricted diffusional space between the plasma membrane and junctional SR elements. Thus myogenic tone and subsequent agonist-induced contraction can be enhanced by treatment with relatively low doses of ouabain, acting to inhibit its target, the Na^+,K^+-ATPase [9]. This inhibition raises sub-sarcolemmal [Na^+], which in turn inhibits extrusion of Ca^{2+} through the Na^+/Ca^{2+} exchanger. Under these conditions of reduced plasma membrane efflux, entering Ca^{2+} is sequestered into the SR stores, leading to an enhanced contractile response.

Many agonists stimulate sustained contraction through the activation of receptor- and store-operated channels that allow both Ca^{2+} and Na^+ entry. Inhibition of the Na^+/Ca^{2+} exchanger under these conditions dramatically reduces agonist responsiveness, suggesting that reverse-mode operation of the exchanger stimulated by elevated Na^+ entering through the store-operated channels, is critical for Ca^{2+} entry, store loading and contraction [168–170]. Despite the fascinating localization of NCX1 demonstrated in smooth muscle cells, and its importance to the physiological role of the exchanger, little specific information has emerged regarding the molecular mechanism for restricted localization, nor details of the macromolecular complex which presumably exists in smooth muscle cells, as well as in other tissues.

The Na^+/Ca^{2+} exchanger plays a special role in Ca^{2+} homoeostasis in the kidney. The majority of Ca^{2+} filtered at the glomerulus is reabsorbed passively along the proximal nephron and in the loop of Henle. However, control of the final few percent of Ca^{2+} reabsorption within the distal nephron is a key point of regulation. It is at this site that hormones such as vitamin D_3 and parathyroid hormone have their action, co-ordinately regulating the apical entry, cytosolic binding and basolateral efflux components of the transepithelial Ca^{2+} transport machinery [171]. Distal nephron segments, particularly the connecting segment, express a very high level of NCX1 on their basolateral membranes [112], which helps to drive transcellular active reabsorption of Ca^{2+}. Thus, in the kidney, NCX1 is involved in the regulation of systemic Ca^{2+} levels, as opposed to regulating the intracellular level of [Ca^{2+}], as is the case in all other tissues in which it is expressed.

Gene-knockout technology has also been applied to the other NCX family members. *NCX2*-knockout mice display enhanced synaptic plasticity and a shift from long-term depression toward long-term potentiation at hippocampal synapses and enhanced performance in several hippocampal-dependent learning and memory tasks [7]. These results are consistent with a predominant role for NCX2 in both pre- and post-synaptic Ca^{2+} clearance. Larger presynaptic [Ca^{2+}] transients due to

the loss of NCX2 function will induce increased transmitter release, whereas similarly increased post-synaptic transients favour the Ca^{2+}-dependent phosphorylation events underlying LTP (long-term potentiation) [172,173]. Presumably other transport pathways account for longer-term Ca^{2+} homoeostasis, which appears normal in these mice.

NCX3-knockout mice have a distinct pathology restricted to deficits in neuromuscular transmission as well as to increased necrosis of muscle tissue [174]. This finding is consistent with the known expression of NCX3 in skeletal muscle. It is not clear whether, in addition to the observed skeletal muscle defects, *NCX3*-knockout mice also display altered neuromuscular control as a result of loss of NCX3 expression in the cerebellum.

NCX1 AND PATHOLOGY

As a consequence of its central role in helping to orchestrate normal cellular Ca^{2+} homoeostasis in a variety of tissues, the Na^+/Ca^{2+} exchanger is also a key player in pathological situations that involve dysregulation of Ca^{2+} balance. Cardiac arrhythmias are a leading cause of death in cardiovascular disease. NCX1 contributes to cardiac arrhythmias in two ways [175,176]. First, reduced forward-mode Ca^{2+} efflux, increased reverse-mode Ca^{2+} influx, or both during systole, mediated by events such as elevated intracellular $[Na^+]$ or other pathogenic mechanisms, can shift the Ca^{2+} extrusion/sequestration balance, leading to SR Ca^{2+} overload. This causes an increase in both the sensitivity and the extent of SR Ca^{2+} release, generating so-called spontaneous release and extrasystolic events [177]. Secondly, the extra Ca^{2+} release increases subsequent NCX1 activity during diastole which will generate an inward current. This depolarizing current can contribute to an elongation of the action potential, or can be of sufficient magnitude to generate extra action potential events.

Heart damage or chronic hypertension can lead to cardiac hypertrophy which eventually results in heart failure. The genetic pathways that are initially activated under these pathological conditions result in an up-regulation of NCX1 [80]. There has been much discussion as to whether these compensatory changes are adaptive or maladaptive to heart function in the long run [178]. It is probable that the initial reprogramming is a response to increased contractile demand that leads to enhanced Ca^{2+} entry and consequent overloading of the SR. An increase in Na^+/Ca^{2+} exchanger extrusion activity will offset the increased Ca^{2+} entry to maintain Ca^{2+} homoeostasis. Eventually, the associated depolarizing current may become arrhythmogenic [179]. Moreover, sustained hypercontractility may cause readjustment of SR Ca^{2+} transport to help reset content. At that point, enhanced Na^+/Ca^{2+} exchanger activity becomes maladaptive, unloading the SR stores which leads to contractile insufficiency and heart failure [180].

The Na^+/Ca^{2+} exchanger is also thought to play a key role in ischaemia/reperfusion injury. Although mechanistically complex with a variety of factors that are likely to contribute to the pathology, it seems likely that H^+ ions that accumulate during the ischaemic period are removed during reperfusion, largely through the action of the Na^+/H^+ exchanger. This transport step leads to a build up of intracellular Na^+, which subsequently either reduces NCX1-mediated Ca^{2+} efflux, or increases Ca^{2+} entry [9,181]. Either way, the consequence is SR Ca^{2+} overloading, and consequent spontaneous release and extrasystolic arrhythmias, as described above.

Action of the Na^+/Ca^{2+} exchanger has also been linked to pathology outside the heart. There is a lot of evidence suggesting that, during excitotoxic stimulation in brain, particularly during ischaemia, large changes in both membrane potential and ion gradients lead to unregulated Ca^{2+} entry and activation of a number of Ca^{2+}-sensitive degradative enzymes, such as the protease calpain, subsequently leading to neuronal degeneration [182,183]. The role of the exchanger in this process has been controversial. On theoretical grounds, one might anticipate that the collapse of the membrane potential and ion gradients would lead to reverse-mode Na^+/Ca^{2+} exchanger-mediated Ca^{2+} entry [184–186]. Under these conditions, inhibition of the exchanger would be expected to protect against ischaemic damage. Indeed, inhibition studies in white matter, and in neurons *in vitro* support such a role. On the other hand, at a later time, during potential recovery from ischaemia particularly in areas penumbral to the major injury, the Na^+/Ca^{2+} exchanger would be anticipated to be a major pathway for clearance of Ca^{2+} that had built up previously. Several animal studies support this neuroprotective role for the exchanger [187–189].

Evidence has been presented that the Na^+/Ca^{2+} exchanger in vascular smooth muscle cells plays an important contributory role to the increase in myotonic tone that underlies salt-sensitive hypertension [190]. Both pharmacological and genetic inhibition of NCX1 were protective against, while transgenic overexpression of NCX1 potentiated, the effect of high salt on hypertension. These data suggest that reverse-mode NCX1 Ca^{2+} entry, possibly in response to Na^+ loading of vascular smooth muscle cells, plays a critical role in the development of salt-sensitive hypertension.

One of the common threads that emerges from examining the physiological and pathophysiological roles for the Na^+/Ca^{2+} exchanger is that altered membrane potential and/or changed ion gradients can lead to unregulated reverse-mode operation of the exchanger, Ca^{2+} overload and deleterious consequences. Forward-mode operation of the exchanger under these conditions is actually protective. Thus consideration of the Na^+/Ca^{2+} exchanger as a putative therapeutic target would require selective inhibition of reverse-mode, as opposed to forward-mode, operation. Coincidentally, over the course of the last 10 years, an expanding family of inhibitors with just these properties have emerged: KB-R7943, SEA0400 and SN-6 [147,191]. Directional inhibition of the exchanger under near equilibrium conditions is, of course, impossible as it violates thermodynamic principles of microreversibility and energy transfer. Apparent directional inhibition can occur far from equilibrium, where one set of conditions (for example those driving reverse-mode operation) favour binding of the inhibitor, while other conditions driving the opposite direction of transport disfavour inhibitor binding [192]. All three inhibitors noted above interact to promote the Na^+-dependent inactivation step of the NCX1 reaction cycle. The inactivated state can only be appreciably populated under conditions favouring reverse-mode operation (high cytosolic $[Na^+]$; relatively low cytosolic $[Ca^{2+}]$), which thus provides a potential explanation for the apparent mode-selective directional inhibition by these compounds. Several previous studies have employed the reverse-mode inhibitors, and demonstrated their effectiveness in providing partial protection against ischaemic damage and hypertension [147,190,191]. These new findings have reinvigorated exploration of the NCX gene family members as targets for therapeutic intervention, and so new more effective and selective classes of inhibitors may be developed in the near future.

THE SLC24 K^+-DEPENDENT Na^+/Ca^{2+} EXCHANGER FAMILY

The protein product in the SLC24, or Na^+/Ca^{2+}–K^+ exchanger, gene family to be identified was NCKX1 in the outer segments of rod photoreceptors [15]. Indeed, NCKX1 remains the only member of the family to have been extensively studied in its

endogenous setting [193]. Following the isolation and cloning of *NCKX1* from bovine retina, the other family members, *NCKX2–NCKX5*, were identified by homology cloning or database mining [22–25]. Sequence comparison among the NCKX family gene products reveals that NCKX1/NCKX2 and NCKX3/NCKX4 pairs each share approx. 60 % amino acid identity, whereas there is only approx. 40 % identity between pairs. NCKX5 is slightly closer in sequence to the NCKX3/NCKX4 pair. In all cases the highest similarity is restricted to the hydrophobic regions of the proteins. A striking difference in length exists between NCKX1 (approx. 1200 amino acids) and the other family members (ranging in length from 500 to 670 amino acids), the extra sequence corresponding to regions of the two large hydrophilic loops in the protein (see below). The apparent size of the NCKX proteins observed on gels also varies with their size, glycosylation state and alternative splicing pattern: ~220 kDa for NCKX1, ~75 kDa for NCKX2, ~70 kDa for NCKX3, ~60 kDa for NCKX4 and ~50 kDa for NCKX5 [14,22–24] (X.-F. Li, F. Visser, G. Yang and J. Lytton, unpublished work). NCKX1, NCKX2 and NCKX4 all have a short segment of their cytosolic loop that is subject to alternative splicing. The role of this splicing event remains unknown, however.

The expression of *NCKX1* is restricted almost exclusively to the eye, although its presence in cells of haemopoietic origin has also been reported [194–196]. *NCKX2* expression is abundant in neurons throughout the brain, with particularly high levels found in deeper cortical layers, hippocampal pyramidal cells, specific thalamic nuclei, the pontine nucleus and the molecular layer and the deep nuclei of the cerebellum [22,195]. *NCKX2* is also expressed in retinal ganglion cells and in cone photoreceptors [197,198]. *NCKX3* and *NCKX4* were also highly expressed in brain, with distinct patterns compared with *NCKX2*, particularly in cortex and thalamus [23,24,195]. These two genes were also more broadly expressed in tissues outside the brain. *NCKX5* was most abundant in the eye and skin, but also expressed at a significant level in brain and thymus, and at much lower levels in other tissues [25]. In contrast with the studies on *NCX1*, there are no reports which study the promoter regions of the NCKX gene family or regulation of expression at the transcriptional level. Molecular cloning studies and database analysis have revealed some heterogeneity at the 5′-end of *NCKX2* transcripts, suggesting multiple possible 5′-untranslated region exons, similar to those found in the *NCX1* gene [22,199]. The significance of such alternative first exons in the *NCKX2* gene remains unknown.

STRUCTURE OF NCKX PROTEINS

The overall topology of the NCKX proteins is identical between family members, and reminiscent of the NCX proteins (see Figure 5). An initial hydrophobic region is thought to be cleaved off as a signal peptide [200], followed by a relatively long extracellular domain containing at least one glycosylation site, a cluster of five proposed hydrophobic transmembrane spans, a larger cytosolic loop and finally a second cluster of hydrophobic transmembrane spans. Experimental evidence places the C-terminus of NCKX2, and by comparison all other NCKX family proteins, outside the cell [38,201]. This is in contrast with the available data for NCX1 which places the C-terminus inside the cell. Thus the C-terminal hydrophobic region may have a different orientation in the NCKX family compared with the NCX family. It is tempting to speculate that this might be related to their different ionic specificities (see below).

The two defining α-repeat regions in NCKX2 have been determined to have opposite orientations with respect to the membrane, much like in NCX1 [37]. There is, however, no evidence in NCKX2 for the re-entrant loop structures observed in NCX1. Proximity analysis using cross-linking experiments provided evidence that the two α-repeat regions are probably close together in the membrane. Just as with NCX1, however, the data were hard to reconcile with a purely helical model [39]. Functional analysis of the α-repeat regions by mutagenesis revealed that many amino acids have an impact on function and on the interaction with the transported ions [32]. Most of these appeared to influence Ca^{2+} and K^+ binding in parallel, suggesting a general influence on the transport mechanism [202]. Several residues stood out from this analysis (see Figure 5). There were two key conserved acid amino acids, glutamic acid in the first α-repeat and aspartic acid in the second, that were absolutely required for transport function. Further analysis has revealed an aspartic acid in the second α-repeat of NCKX family members that when altered to the corresponding asparagine residue found in the NCX family, rendered the NCKX family K^+-independent [203]. Presumably this residue is critical in defining the K^+-binding site.

NCKX TRANSPORT FUNCTION

Recent analysis comparing ion binding with the external facing transport sites between different NCKX family members has demonstrated a similar apparent affinity of approx. 1 μM for Ca^{2+} and approx. 30 mM for Na^+ [204]. Na^+ activation of transport displayed a co-operative interaction, consistent with three or more ions binding, whereas both Ca^{2+} and K^+ displayed activation consistent with a single ion binding. In the case of K^+, when analysed in the presence of Li^+ as the counterion, NCKX2 displayed a dramatically lower apparent affinity of approx. 40 mM compared with approx. 1 mM for NCKX3 or NCKX4. Selectivity for other ions at the K^+ site of NCKX2 was also significantly different, with both Cs^+ and NH_4^+ stimulating transport to a greater extent than in NCKX3 or NCKX4 [205]. Mutation of the α-repeat residues that differ between NCKX2 and NCKX4 revealed that a threonine residue in the second α-repeat of NCKX2, lying just cytoplasmic to the key conserved aspartic acid, was responsible for the low apparent K^+ affinity of NCKX2 [204]. Thus mutation of this single residue to the corresponding alanine residue in NCKX4 converted NCKX2 from low into high apparent K^+ affinity. Less is known regarding ion binding from the cytoplasmic side of the membrane, although some studies in rod outer segments suggest that both Ca^{2+} and K^+ interact with similar apparent affinity to their binding sites from both sides of the membrane [206]. The physiological significance of the difference in apparent K^+ affinity between isoforms is not obvious, but may relate to differential localization of the isoforms at sites of $[K^+]$ fluctuation.

REGULATION OF NCKX FUNCTION

Information on regulation of NCKX family members is relatively under-developed compared with what is known for NCX1. Activity of the latter is regulated via the central cytosolic domain, and so it seems reasonable to begin exploring NCKX regulation by examining the corresponding region of those proteins. Unlike the NCX family, there is little sequence similarity in the central cytosolic loop of NCKX family members. Both NCKX1 and NCKX2 have a short amphipathic region at the N-terminal end of the central cytosolic loop, but it does not resemble the XIP region of NCX1 significantly. In addition, just downstream of this region, NCKX1 and NCKX2 share a short stretch of amino acid similarity surrounding a possible CaMKII (Ca^{2+}/calmodulin-dependent protein kinase) phosphorylation consensus motif [22].

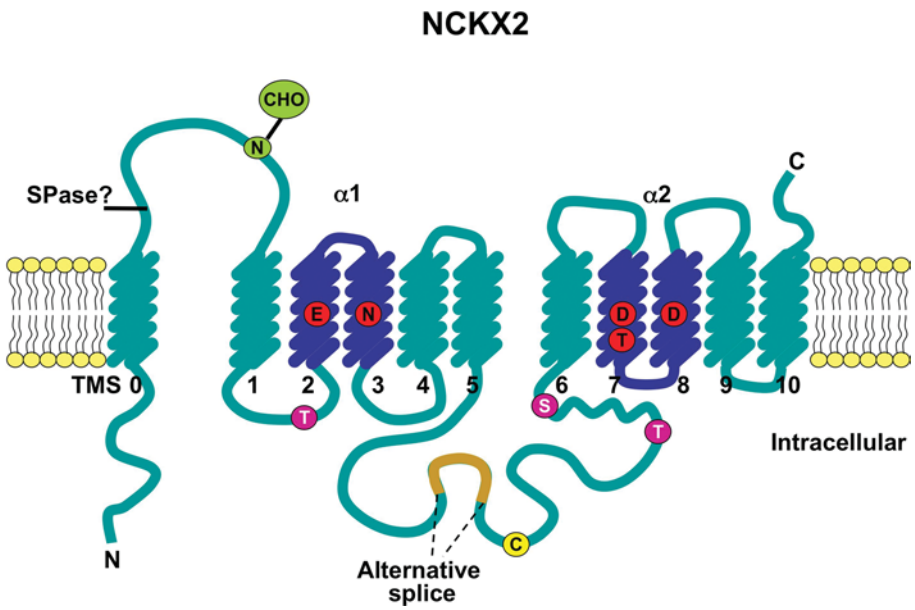

Figure 5 Topology model for NCKX2

The experimentally supported topology model for NCKX2 has ten transmembrane-spanning segments (TMSs), a cleaved 'signal peptide' (TMS0, with the putative signal peptide cleavage site shown as SPase?), but no re-entrant loops. The conserved α-repeat motifs are shown in dark blue (labelled α1 and α2), and the single N-linked glycosylation site is shown in green (N-CHO). Critical amino acids in the α-repeat regions are highlighted in red (Glu^{188}, Asn^{215}, Asp^{548}, Thr^{551} and Asp^{575}). In the cytosolic loop, the region subject to alternative splicing is indicated, as well as three consensus PKC phosphorylation sites thought to be involved in regulation of activity (Thr^{166}, Thr^{476} and Ser^{504}) and a cysteine residue (Cys^{395}) thought to be important for redox regulation of NCKX2 activity and higher-order oligomer formation.

Neither of these regions has been examined experimentally to see whether it serves a regulatory role.

The NCKX1 cytosolic loop contains an unusual repeated acidic amino acid motif. Although this general feature is found in common between *NCKX1* gene products from different species, the length of the motif, its sequence and the number of times it is repeated are not conserved among rats, cows or humans [196]. A similar acidic repeat motif (although not of similar sequence) is present in a subunit of the cGMP-gated channel [207], which is found to associate with NCKX1 in a molecular complex in rod photoreceptors [208]. The role of the acidic motifs in complex formation has not been tested. The NCKX1/cGMP-gated channel complex may be important for the co-ordinated regulation of cytosolic [Ca^{2+}] in the rod outer segment. There is also evidence for complex formation between cGMP-gated channel subunits and both NCKX1 and NCKX2, when studied using cross-linking in recombinant expression systems [209].

Both NCKX1 and NCKX2 form higher-order oligomeric structures when expressed in HEK-293 (human embryonic kidney) cells [201,209,210]. The presence of the higher-molecular-mass cross-linked species is dependent upon the presence of single cysteine residues: in the extracellular loop in NCKX1 and in the central cytosolic loop in NCKX2 [209]. Interestingly, for NCKX2, the size of the cross-linked product is much greater than predicted for a dimer, suggesting a structure with an aberrant mobility, a higher-order oligomer or an unidentified interaction partner. Under resting conditions, the cytosolic cysteine residue in the central loop of NCKX2 is not accessible unless subject to prior reduction, suggesting that it may normally be occupied by a small molecule or engaged in a disulfide bond. Reduction of this cysteine leads to modest activation of the exchanger, possibly by relief of some steric hindrance [201]. These data are suggestive of protein–protein interactions that may have important regulatory influences on NCKX function. Further work is clearly needed to resolve this issue.

Measurements of NCKX2 function in heterologous systems typically involve loading cells with Na^+ to various extents and then following Ca^{2+} entry with a fluorescent dye trapped in the cytoplasm. When a gramicidin-clamp was used to control the [Na^+] at defined levels, a time-dependent inactivation process was revealed that required high levels of intracellular [Na^+], apparently via a mechanism involving binding to the transport sites [211]. This process is reminiscent of the Na^+-dependent inactivation that has been well described for NCX1 (see above).

Regulation of NCKX function by phosphorylation via PKC has been demonstrated recently for NCKX2, but was not observed in either NCKX3 or NCKX4 [212]. The neurotransmitter glutamate acting through synaptic metabotropic receptors would be expected to induce a similar PKC-mediated activation [213,214]. Indeed, activation of NCKX transport by phorbol ester could be demonstrated at the large presynaptic terminal, the calyx of Held. Treatment of NCKX2-transfected HEK-293 cells with phorbol ester resulted in significant stimulation of exchange activity, which could be blocked by overexpression of dominant-negative PKCε. Stimulation of activity correlated with an increase in the level of incorporation of [^{32}P]phosphate into immunoprecipitated NCKX2, and could be blocked by mutation of multiple potential PKC phosphorylation sites on NCKX2, but not by any one single mutation (see Figure 5). These data suggest a complex model of regulation involving direct phosphorylation of NCKX2. These new modes of regulation of NCKX2 may play an important role in mediating complex integration of Ca^{2+} at synaptic sites, where rapid changes in Ca^{2+} flux play important roles in the control of neurotransmitter release and in the development of synaptic plasticity.

NCKX AND PHYSIOLOGY

NCKX transport function has been best described in the rod photoreceptor outer segments, where NCKX1 is the principal

means for Ca^{2+} extrusion [193]. Here, in the dark, cGMP-gated channels are largely open, which results in a depolarizing Na^+ entry as well as Ca^{2+} influx that is matched by NCKX1-mediated efflux. Under these conditions, the thermodynamic and kinetic properties of an NCKX-type transporter are critical for the maintenance of Ca^{2+} homoeostasis. Upon stimulation with light, cGMP is hydrolysed, the cGMP-gated channels close, the resulting hyperpolarization triggering a change in signalling at the photoreceptor synapse, and NCKX1 activity drives Ca^{2+} down to a very low level. This change in [Ca^{2+}] is a key mediator of visual adaptation, resulting in an increase in cGMP synthesis and re-opening of the cGMP-gated channels under continual illumination [207,215]. NCKX2 likely plays a corresponding role in cone photoreceptors [198].

Discovery of the other NCKX family members originated in molecular cloning work, and so functional knowledge has lagged behind structural information. Recent findings, in axonal termini from the posterior pituitary, as well as at the giant synapse of the calyx of Held, have demonstrated a prominent role for K^+-dependent Na^+/Ca^{2+} exchange in Ca^{2+} extrusion, particularly when intracellular [Ca^{2+}] is high [3,4,216]. In addition, studies on hippocampal neurons demonstrate a large component of NCKX-mediated Ca^{2+} flux [217]. On the basis of similar considerations of thermodynamics, kinetics and the parallel to the rod photoreceptors, it seems likely that NCKX expression at these sites allows the maintenance of Ca^{2+} extrusion and homoeostasis in neuronal environments which experience large and frequent ionic fluctuations across the plasma membrane. None of these studies, however, could distinguish which of the possible NCKX isoforms present (NCKX2, NCKX3 or NCKX4) was responsible for the activities measured.

The *NCKX5* gene was recently found to have an unexpected role in the development of pigmentation in skin and retinal epithelia [25]. Analysis of the 'golden' phenotype in zebrafish identified a mutation in the *NCKX5* gene resulting in impaired skin pigmentation. This defect could be partially rescued by reintroduction of wild-type zebrafish or human *NCKX5* genes. Furthermore, analysis of the SNP (single nucleotide polymorphism) databases revealed a striking association of a single coding region polymorphism in *NCKX5*, changing a highly conserved alanine to a threonine residue, with humans of Northern European origin, in comparison with those of African or Asian origin. Analysis of the NCKX5 protein in melanoma cells suggests that it associates with mature melanosomes [25,218]. This would be a unique cellular localization for NCKX5, as other members of the NCKX family are found at the plasma membrane. It has been hypothesized that, in the melanosome, NCKX5 may be coupled to the proton gradient generated by the V-ATPase (vacuolar-type ATPase) via a Na^+/H^+ exchanger and thus drive Ca^{2+} uptake [25]. However, the role for the NCKX5 protein or for Ca^{2+} transport in melanosome biogenesis and maturation is not known. Neither is there any information concerning the impact on function of the polymorphism associated with European populations. Furthermore, significant levels of *NCKX5* mRNA have been found in brain and thymus, although neither localization nor protein expression has been investigated in those tissues [25]. NCKX5 function is thus a fertile and exciting new area of investigation.

Although the physiological role for NCKX1 in rod and NCKX2 in cone photoreceptors, and the probable role for NCKX5 in melanocytes has become clear over the last few years, specific and distinct roles for NCKX2, NCKX3 and NCKX4 are only beginning to be unravelled. These three gene products are widely expressed in brain neurons, along with exchangers of the NCX family, NCX1, NCX2 and NCX3. Distinct patterns of neuronal expression have been described for all of these gene products, although no clear physiological insight has yet emerged from those studies [195]. In addition to brain, functional evidence is beginning to emerge for an important role of NCKX-type exchangers in other cells and tissues, such as olfactory epithelium [219], vestibular sensory cells [220], smooth muscle cells [168] and spermatozoa [221].

More recently, the *NCKX2* gene has been knocked out in mice with interesting consequences [8]. These animals display a clear deficit in neuronal Ca^{2+} transport and no overt compensatory increases in other known Ca^{2+} extrusion or sequestration pathways. However, there is a dramatic loss of LTP and a gain in long-term depression at hippocampal Shaffer collateral/CA1 synapses. This result is anticipated for the loss of a postsynaptic extrusion mechanism, leading to a sustained Ca^{2+} elevation that shifts the balance from kinase to phosphatase activation [172,173]. The phenotype of the *NCKX2*-knockout mice is also in marked contrast with that of the *NCX2*-knockout mice [7]. The differences may in part be due to pre- compared with post-synaptic expression, but may also reflect kinetic differences in operation, such that *NCX2* knockout predominantly affects the magnitude of the initial Ca^{2+} transient, whereas *NCKX2* knockout predominantly influences the sustained Ca^{2+} response. Such a dramatic change in function in cells that express more than one Na^+/Ca^{2+} exchanger gene product clearly demonstrates the non-redundant and distinct actions of the different transporters. Although the effect of NCKX2 knockout on synaptic plasticity in the hippocampus was dramatic, the influence of these cellular changes on behaviour was relatively subtle. The *NCKX2*-knockout mice displayed no overt behavioural abnormalities, including classical tests of motor skill and memory. There was, however, a clear deficit in performance on specific tests of motor learning and memory that depended upon the benefit of prior experience. It will be exciting to continue investigating the mechanisms that underlie these distinct functional deficits in *NCKX2*-knockout mice and to anticipate specific changes resulting from knockout of the other NCKX gene family members.

NCKX AND PATHOPHYSIOLOGY

To date, no genetic disease has been clearly linked by either inheritance or association with any Na^+/Ca^{2+} exchanger gene in either the NCX or NCKX family. There is a significant ongoing effort to identify genetic causes of retinal disease, including screening for possible mutations in *NCKX1* and *NCKX2* [199]. Several polymorphisms were identified in this study, some of which were considered to be pathogenic, especially in *NCKX1*. However, no definitive linkages could be confirmed.

The question of whether NCKX isoforms are neuroprotective in the face of ischaemic and/or excitotoxic insults has recently been a focus of several studies. Following a short period of ischaemia, ion gradients and membrane potential collapses precipitously, pH drops and uncontrolled glutamate release activates further cation fluxes [222]. These are all changes which, in principle, might be sufficient to reverse the direction of even NCKX family members. Studies using *in vitro* cultures exposed to altered ionic conditions, while inhibiting various Na^+/Ca^{2+} exchange activities supports a contributing role for both NCX and NCKX gene products to excitotoxic Ca^{2+} accumulation and neuronal damage [186,223]. On the other hand, animal models examining changing levels of expression and application of siRNA (small interfering RNA) to knockdown expression of selective NCX and NCKX members supports a protective role for NCKX2 in the development of ischaemic injury [224]. Clearly, further careful studies will be required to resolve these issues, and to define a time course for the relative action of different Na^+/Ca^{2+} exchangers in the

development of ischaemic damage. This is an important area of study that may provide new targets for therapeutic intervention in excitotoxic disease states.

THE CCX EXCHANGER FAMILY

The CCX branch of the CaCA exchanger superfamily contains one mammalian member, so called NCKX6, or NCLX [26,27]. This most recently cloned member of the family, which is widely expressed at moderate levels, was initially mis-classified as part of the NCKX branch. However, recognition that its sequence was significantly more divergent than other NCKX family members led to a more detailed phylogenetic study and reclassification [17]. Membership in a distinct clade of the CaCA superfamily tree implies that NCKX6/NCLX probably has distinct endogenous substrate specificity. Indeed, Palty et al. [27] suggested that Li^+ can substitute for Na^+ in this exchanger, to mediate K^+-independent Li^+/Ca^{2+} exchange. It seems unlikely, however, that Li^+ is the endogenous substrate for this transporter. Intriguingly, full-length NCKX6/NCLX is retained in an endoplasmic reticulum-like compartment when expressed in HEK-293 cells [26]. If this is its endogenous site of expression (as is the case for NCKX5), NCKX6/NCLX may play a role in loading a novel Ca^{2+} store. An intriguing finding of studies on recombinant NCKX6/NCLX was that partial-length truncated proteins, containing only a single copy of the α-repeat, appeared to be functional [26,27]. This phenomenon of active partial length exchangers has also been reported for NCX1 [225,226]. Dimerization of these proteins may account for their function [227]. However, as it is now fairly well established that the two copies of the α-repeats present in a single polypeptide of the CaCA superfamily have opposite orientations with respect to the membrane [228], it is unclear how a homodimer of a half protein would be functional. Possible explanations might involve an unidentified endogenous partner, and/or the concept of dual-topology precursors to the CaCA superfamily [40]. In the latter model, either the truncated half NCKX6/NCLX molecules are competent to incorporate into the membrane in two orientations and then associate as dimers, or a second unidentified partner, in the opposite orientation to the truncated half NCKX6/NCLX molecule, combines to form a (partially) active transporter. The new class of CCX transporters clearly warrants further examination.

CONCLUDING REMARKS

Since the initial molecular cloning of *NCX1* and *NCKX1* in the early 1990s, there has been a large expansion of structural information regarding Na^+/Ca^{2+} exchangers, resulting in the identification of the CaCA superfamily, and a realization that Na^+/Ca^{2+} exchanger function is encoded by a complex array of different gene products. The NCX1 protein of cardiac myocytes is clearly the best studied of these, yet even this protein yields new surprises every year. Similarly, members of the NCKX family continue to surprise with their much broader and varied roles than originally appreciated. The recently published proceedings from the Fifth International Conference on Sodium–Calcium Exchange are an excellent source of current information [229]. Full characterization of the complex functional and regulatory properties of all the different proteins, and a clearer understanding of the physiological role(s) for each, is a work in progress. These studies are anticipated to reveal intriguing new distinct roles for different Na^+/Ca^{2+} exchanges in the control of Ca^{2+} transport and homoeostasis in a variety of cellular environments.

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REFERENCES

1 Berridge, M. J., Lipp, P. and Bootman, M. D. (2000) The versatility and universality of calcium signalling. Nat. Rev. Mol. Cell Biol. **1**, 11–21
2 Berridge, M. J., Bootman, M. D. and Roderick, H. L. (2003) Calcium signalling: dynamics, homeostasis and remodelling. Nat. Rev. Mol. Cell Biol. **4**, 517–529
3 Lee, S. H., Kim, M. H., Park, K. H., Earm, Y. E. and Ho, W. K. (2002) K^+-dependent Na^+/Ca^{2+} exchange is a major Ca^{2+} clearance mechanism in axon terminals of rat neurohypophysis. J. Neurosci. **22**, 6891–6899
4 Kim, M. H., Korogod, N., Schneggenburger, R., Ho, W. K. and Lee, S. H. (2005) Interplay between Na^+/Ca^{2+} exchangers and mitochondria in Ca^{2+} clearance at the calyx of Held. J. Neurosci. **25**, 6057–6065
5 Wanaverbecq, N., Marsh, S. J., Al-Qatari, M. and Brown, D. A. (2003) The plasma membrane calcium-ATPase as a major mechanism for intracellular calcium regulation in neurones from the rat superior cervical ganglion. J. Physiol. **550**, 83–101
6 Pott, C., Henderson, S. A., Goldhaber, J. I. and Philipson, K. D. (2007) Na^+/Ca^{2+} exchanger knockout mice: plasticity of cardiac excitation–contraction coupling. Ann. N.Y. Acad. Sci. **1099**, 270–275
7 Jeon, D., Yang, Y. M., Jeong, M. J., Philipson, K. D., Rhim, H. and Shin, H. S. (2003) Enhanced learning and memory in mice lacking Na^+/Ca^{2+} exchanger 2. Neuron **38**, 965–976
8 Li, X. F., Kiedrowski, L., Tremblay, F., Fernandez, F. R., Perizzolo, M., Winkfein, R. J., Turner, R. W., Bains, J. S., Rancourt, D. E. and Lytton, J. (2006) Importance of K^+-dependent Na^+/Ca^{2+}-exchanger 2, NCKX2, in motor learning and memory. J. Biol. Chem. **281**, 6273–6282
9 Blaustein, M. P. and Lederer, W. J. (1999) Sodium/calcium exchange: its physiological implications. Physiol. Rev. **79**, 763–854
10 Nicoll, D. A., Longoni, S. and Philipson, K. D. (1990) Molecular cloning and functional expression of the cardiac Na,Ca-exchanger. Science **250**, 562–565
11 Schnetkamp, P. P. M. (1986) Sodium–calcium exchange in the outer segments of bovine rod photoreceptors. J. Physiol. **373**, 25–45
12 Cervetto, L., Lagnado, L., Perry, R. J., Robinson, D. W. and McNaughton, P. A. (1989) Extrusion of calcium from rod outer segments is driven by both sodium and potassium gradients. Nature **337**, 740–743
13 Schnetkamp, P. P. M., Basu, D. K. and Szerencsei, R. T. (1989) Na^+–Ca^{2+} exchange in bovine rod outer segments requires and transports K^+. Am. J. Physiol. Cell Physiol. **257**, C153–C157
14 Cook, N. J. and Kaupp, U. B. (1988) Solubilization, purification, and reconstitution of the sodium–calcium exchanger from bovine retinal rod outer segments. J. Biol. Chem. **263**, 11382–11388
15 Reiländer, H., Achilles, A., Friedel, U., Maul, G., Lottspeich, F. and Cook, N. J. (1992) Primary structure and functional expression of the Na/Ca,K-exchanger from bovine rod photoreceptors. EMBO J. **11**, 1689–1695
16 Schwarz, E. M. and Benzer, S. (1997) *Calx*, a sodium–calcium exchanger gene of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. U.S.A. **94**, 10249–10254
17 Cai, X. and Lytton, J. (2004) The cation/Ca^{2+} exchanger superfamily: phylogenetic analysis and structural implications. Mol. Biol. Evol. **21**, 1692–1703
18 Ren, Q., Chen, K. and Paulsen, I. T. (2007) TransportDB: a comprehensive database resource for cytoplasmic membrane transport systems and outer membrane channels. Nucleic Acids Res. **35**, D274–D279
19 Li, Z., Matsuoka, S., Hryshko, L. V., Nicoll, D. A., Bersohn, M. M., Burke, E. P., Lifton, R. P. and Philipson, K. D. (1994) Cloning of the NCX2 isoform of the plasma membrane Na–Ca exchanger. J. Biol. Chem. **269**, 17434–17439
20 Nicoll, D. A., Quednau, B. D., Qui, Z., Xia, Y.-R., Lusis, A. J. and Philipson, K. D. (1996) Cloning of a third mammalian Na^+–Ca^{2+} exchanger, NCX2. J. Biol. Chem. **271**, 24914–24921
21 Marshall, C. R., Pan, T. C., Le, H. D., Omelchenko, A., Hwang, P. P., Hryshko, L. V. and Tibbits, G. F. (2005) cDNA cloning and expression of the cardiac Na^+/Ca^{2+} exchanger from Mozambique tilapia (*Oreochromis mossambicus*) reveal a teleost membrane transporter with mammalian temperature dependence. J. Biol. Chem. **280**, 28903–28911
22 Tsoi, M., Rhee, K.-H., Bungard, D., Li, X.-F., Lee, S.-L., Auer, R. N. and Lytton, J. (1998) Molecular cloning of a novel potassium-dependent sodium–calcium exchanger from rat brain. J. Biol. Chem. **273**, 4155–4162

23 Kraev, A., Quednau, B. D., Leach, S., Li, X. F., Dong, H., Winkfein, R., Perizzolo, M., Cai, X., Yang, R., Philipson, K. D. and Lytton, J. (2001) Molecular cloning of a third member of the potassium-dependent sodium–calcium exchanger gene family, NCKX3. J. Biol. Chem. **276**, 23161–23172

24 Li, X. F., Kraev, A. S. and Lytton, J. (2002) Molecular cloning of a fourth member of the potassium-dependent sodium–calcium exchanger gene family, NCKX4. J. Biol. Chem. **277**, 48410–48417

25 Lamason, R. L., Mohideen, M. A., Mest, J. R., Wong, A. C., Norton, H. L., Aros, M. C., Jurynec, M. J., Mao, X., Humphreville, V. R., Humbert, J. E. et al. (2005) SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. Science **310**, 1782–1786

26 Cai, X. and Lytton, J. (2004) Molecular cloning of a sixth member of the K^+-dependent Na^+/Ca^{2+} exchanger gene family, NCKX6. J. Biol. Chem. **279**, 5867–5876

27 Palty, R., Ohana, E., Hershfinkel, M., Volokita, M., Elgazar, V., Beharier, O., Silverman, W. F., Argaman, M. and Sekler, I. (2004) Lithium–calcium exchange is mediated by a distinct potassium-independent sodium–calcium exchanger. J. Biol. Chem. **279**, 25234–25240

28 Nicoll, D. A., Hryshko, L. V., Matsuoka, S., Frank, J. S. and Philipson, K. D. (1996) Mutation of amino acid residues in the putative transmembrane segments of the cardiac sarcolemmal Na^+–Ca^{2+} exchanger. J. Biol. Chem. **271**, 13385–13391

29 Iwamoto, T., Uehara, A., Imanaga, I. and Shigekawa, M. (2000) The Na^+/Ca^{2+} exchanger NCX1 has oppositely oriented reentrant loop domains that contain conserved aspartic acids whose mutation alters its apparent Ca^{2+} affinity. J. Biol. Chem. **275**, 38571–38580

30 Ottolia, M., Nicoll, D. A. and Philipson, K. D. (2005) Mutational analysis of the α-1 repeat of the cardiac Na^+-Ca^{2+} exchanger. J. Biol. Chem. **280**, 1061–1069

31 Nicoll, D. A., Ren, X., Ottolia, M., Phillips, M., Paredes, A. R., Abramson, J. and Philipson, K. D. (2007) What we know about the structure of NCX1 and how it relates to its function. Ann. N.Y. Acad. Sci. **1099**, 1–6

32 Winkfein, R. J., Szerencsei, R. T., Kinjo, T. G., Kang, K., Perizzolo, M., Eisner, L. and Schnetkamp, P. P. (2003) Scanning mutagenesis of the α repeats and of the transmembrane acidic residues of the human retinal cone Na/Ca–K exchanger. Biochemistry **42**, 543–552

33 Shibukawa, Y., Kang, K. J., Kinjo, T. G., Szerencsei, R. T., Altimimi, H. F., Pratikhya, P., Winkfein, R. J. and Schnetkamp, P. P. (2007) Structure–function relationships of the NCKX2 Na^+/Ca^{2+}–K^+ exchanger. Ann. N.Y. Acad. Sci. **1099**, 16–28

34 Nicoll, D. A., Ottolia, M., Lu, L., Lu, Y. and Philipson, K. D. (1999) A new topological model of the cardiac sarcolemmal Na^+-Ca^{2+} exchanger. J. Biol. Chem. **274**, 910–917

35 Qiu, Z., Nicoll, D. A. and Philipson, K. D. (2001) Helix packing of functionally important regions of the cardiac Na^+–Ca^{2+} exchanger. J. Biol. Chem. **276**, 194–199

36 Ren, X., Nicoll, D. A. and Philipson, K. D. (2006) Helix packing of the cardiac Na^+–Ca^{2+} exchanger: proximity of transmembrane segments 1, 2, and 6. J. Biol. Chem. **281**, 22808–22814

37 Kinjo, T. G., Szerencsei, R. T. and Schnetkamp, P. P. (2007) Topologic investigation of the NCKX2 Na^+/Ca^{2+}–K^+ exchanger α-repeats. Ann. N.Y. Acad. Sci. **1099**, 34–39

38 Kinjo, T. G., Szerencsei, R. T., Winkfein, R. J., Kang, K. and Schnetkamp, P. P. (2003) Topology of the retinal cone NCKX2 Na/Ca–K exchanger. Biochemistry **42**, 2485–2491

39 Kinjo, T. G., Kang, K., Szerencsei, R. T., Winkfein, R. J. and Schnetkamp, P. P. (2005) Site-directed disulfide mapping of residues contributing to the Ca^{2+} and K^+ binding pocket of the NCKX2 Na^+/Ca^{2+}–K^+ exchanger. Biochemistry **44**, 7787–7795

40 Rapp, M., Granseth, E., Seppala, S. and von Heijne, G. (2006) Identification and evolution of dual-topology membrane proteins. Nat. Struct. Mol. Biol. **13**, 112–116

41 Rapp, M., Seppala, S., Granseth, E. and von Heijne, G. (2007) Emulating membrane protein evolution by rational design. Science **315**, 1282–1284

42 Kofuji, P., Hadley, R. W., Kieval, R. S., Lederer, W. J. and Schulze, D. H. (1992) Expression of the Na–Ca exchanger in diverse tissues: a study using the cloned human cardiac Na–Ca exchanger. Am. J. Physiol. Cell Physiol. **263**, C1241–C1249

43 Lee, S.-L., Yu, A. S. L. and Lytton, J. (1994) Tissue-specific expression of Na/Ca exchanger isoforms. J. Biol. Chem. **269**, 14849–14852

44 Quednau, B. D., Nicoll, D. A. and Philipson, K. D. (2004) The sodium/calcium exchanger family-SLC8. Pflügers Arch. **447**, 543–548

45 Kofuji, P., Lederer, W. J. and Schulze, D. H. (1994) Mutually exclusive and cassette exons underlie alternatively spliced isoforms of the Na/Ca exchanger. J. Biol. Chem. **269**, 5145–5149

46 Barnes, K. V., Cheng, G., Dawson, M. M. and Menick, D. R. (1997) Cloning of cardiac, kidney, and brain promoters of the feline *ncx1* gene. J. Biol. Chem. **272**, 11510–11517

47 Nicholas, S. B., Yang, W., Lee, S. L., Zhu, H., Philipson, K. D. and Lytton, J. (1998) Alternative promoters and cardiac muscle cell-specific expression of the Na^+/Ca^{2+} exchanger gene. Am. J. Physiol. Heart Circ. Physiol. **274**, H217–H232

48 Quednau, B. D., Nicoll, D. A. and Philipson, K. D. (1997) Tissue specificity and alternative splicing of the Na^+/Ca^{2+} exchanger isoforms NCX1, NCX2, and NCX3 in rat. Am. J. Physiol. Cell Physiol. **272**, C1250–C1261

49 Papa, M., Canitano, A., Boscia, F., Castaldo, P., Sellitti, S., Porzig, H., Taglialatela, M. and Annunziato, L. (2003) Differential expression of the Na^+–Ca^{2+} exchanger transcripts and proteins in rat brain regions. J. Comp. Neurol. **461**, 31–48

50 Durkin, J. T., Ahrens, D. C., Pan, Y. C. E. and Reeves, J. P. (1991) Purification and amino-terminal sequence of the bovine cardiac sodium–calcium exchanger: evidence for the presence of a signal sequence. Arch. Biochem. Biophys. **290**, 369–375

51 Furman, I., Cook, O., Kasir, J., Low, W. and Rahamimoff, H. (1995) The putative amino-terminal signal peptide of the cloned rat brain Na^+–Ca^{2+} exchanger gene (RBE-1) is not mandatory for functional expression. J. Biol. Chem. **270**, 19120–19127

52 Loo, T. W., Ho, C. and Clarke, D. M. (1995) Expression of a functionally active human renal sodium–calcium exchanger lacking a signal sequence. J. Biol. Chem. **270**, 19345–19350

53 Sahin-Toth, M., Nicoll, D. A., Frank, J. S., Philipson, K. D. and Friedlander, M. (1995) The cleaved N-terminal signal sequence of the cardiac Na^+-Ca^{2+} exchanger is not required for functional membrane integration. Biochem. Biophys. Res. Commun. **212**, 968–974

54 Hryshko, L. V., Nicoll, D. A., Weiss, J. N. and Philipson, K. D. (1993) Biosynthesis and initial processing of the cardiac sarcolemmal Na^+–Ca^{2+} exchanger. Biochim. Biophys. Acta **1151**, 35–42

55 Cook, O., Low, W. and Rahamimoff, H. (1998) Membrane topology of the rat brain Na^+–Ca^{2+} exchanger. Biochim. Biophys. Acta **1371**, 40–52

56 Secondo, A., Staiano, R. I., Scorziello, A., Sirabella, R., Boscia, F., Adornetto, A., Valsecchi, V., Molinaro, P., Canzoniero, L. M., Di Renzo, G. and Annunziato, L. (2007) BHK cells transfected with NCX3 are more resistant to hypoxia followed by reoxygenation than those transfected with NCX1 and NCX2: possible relationship with mitochondrial membrane potential, Cell Calcium, doi:10.1016/j.ceca.2007.01.006

57 Huang, Y., Lemieux, M. J., Song, J., Auer, M. and Wang, D. N. (2003) Structure and mechanism of the glycerol-3-phosphate transporter from *Escherichia coli*. Science **301**, 616–620

58 Li, Z., Nicoll, D. A., Collins, A., Hilgemann, D. W., Filoteo, A. G., Penniston, J. T., Weiss, J. N., Tomich, J. M. and Philipson, K. D. (1991) Identification of a peptide inhibitor of the cardiac sarcolemmal Na,Ca-exchanger. J. Biol. Chem. **266**, 1014–1020

59 Hilge, M., Aelen, J. and Vuister, G. W. (2006) Ca^{2+} regulation in the Na^+/Ca^{2+} exchanger involves two markedly different Ca^{2+} sensors. Mol. Cell **22**, 15–25

60 Nicoll, D. A., Sawaya, M. R., Kwon, S., Cascio, D., Philipson, K. D. and Abramson, J. (2006) The crystal structure of the primary Ca^{2+} sensor of the Na^+/Ca^{2+} exchanger reveals a novel Ca^{2+} binding motif. J. Biol. Chem. **281**, 21577–21581

61 Linck, B., Qiu, Z., He, Z., Tong, Q., Hilgemann, D. W. and Philipson, K. D. (1998) Functional comparison of the three isoforms of the Na^+/Ca^{2+} exchanger (NCX1, NCX2, NCX3). Am. J. Physiol. Cell Physiol. **274**, C415–C423

62 Reeves, J. P. and Sutko, J. L. (1980) Sodium–calcium exchange activity generates a current in cardiac membrane vesicles. Science **208**, 1461–1464

63 Kimura, J., Miyamae, S. and Noma, A. (1987) Identification of sodium–calcium exchange current in single ventricular cells of guinea-pig. J. Physiol. **384**, 199–222

64 Fujioka, Y., Komeda, M. and Matsuoka, S. (2000) Stoichiometry of Na^+–Ca^{2+} exchange in inside-out patches excised from guinea-pig ventricular myocytes. J. Physiol. **523**, 339–351

65 Dong, H., Dunn, J. and Lytton, J. (2002) Stoichiometry of the cardiac Na^+/Ca^{2+} exchanger NCX1.1 measured in transfected HEK cells. Biophys. J. **82**, 1943–1952

66 Kang, T. M. and Hilgemann, D. W. (2004) Multiple transport modes of the cardiac Na^+/Ca^{2+} exchanger. Nature **427**, 544–548

67 Reeves, J. P. and Sutko, J. L. (1983) Competitive interactions of sodium and calcium with the sodium–calcium exchange system of cardiac sarcolemmal vesicles. J. Biol. Chem. **258**, 3178–3182

68 Bers, D. M. (2002) Cardiac excitation–contraction coupling. Nature **415**, 198–205

69 Lederer, W. J., Niggli, E. and Hadley, R. W. (1990) Sodium–calcium exchange in excitable cells: fuzzy space. Science **248**, 283

70 Lencesova, L., O'Neill, A., Resneck, W. G., Bloch, R. J. and Blaustein, M. P. (2004) Plasma membrane–cytoskeleton–endoplasmic reticulum complexes in neurons and astrocytes. J. Biol. Chem. **279**, 2885–2893

71 Schulze, D. H., Muqhal, M., Lederer, W. J. and Ruknudin, A. M. (2003) Sodium/calcium exchanger (NCX1) macromolecular complex. J. Biol. Chem. **278**, 28849–28855

72 Mohler, P. J., Splawski, I., Napolitano, C., Bottelli, G., Sharpe, L., Timothy, K., Priori, S. G., Keating, M. T. and Bennett, V. (2004) A cardiac arrhythmia syndrome caused by loss of ankyrin-B function. Proc. Natl. Acad. Sci. U.S.A. **101**, 9137–9142

73 Artman, M., Ichikawa, H., Avkiran, M. and Coetzee, W. A. (1995) Na^+/Ca^{2+} exchange current density in cardiac myocytes from rabbits and guinea pigs during postnatal development. Am. J. Physiol. **268**, H1714–H1722

74 Boerth, S. R., Zimmer, D. B. and Artman, M. (1994) Steady-state mRNA levels of the sarcolemmal Na–Ca exchanger peak near birth in developing rabbit and rat hearts. Circ. Res. **74**, 354–359

75 Chen, F., Mottino, G., Klitzner, T. S., Philipson, K. D. and Frank, J. S. (1995) Distribution of the Na^+/Ca^{2+} exchange protein in developing rabbit myocytes. Am. J. Physiol. Cell Physiol. **268**, C1126–C1132

76 Huang, J., Hove-Madsen, L. and Tibbits, G. F. (2005) Na^+/Ca^{2+} exchange activity in neonatal rabbit ventricular myocytes. Am. J. Physiol. Cell Physiol. **288**, C195–C203

77 Tibbits, G. F., Xu, L. and Sedarat, F. (2002) Ontogeny of excitation–contraction coupling in the mammalian heart. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. **132**, 691–698

78 Kent, R. L., Rozich, J. D., McCollam, P. L., McDermott, D. E., Thacher, U. F., Menich, D. R., McDermott, P. J. and Cooper IV, G. (1993) Rapid expression of the Na,Ca-exchanger in response to cardiac pressure overload. Am. J. Physiol. **263**, H1024–H1029

79 Studer, R., Reinecke, H., Bilger, J., Eschenhagen, T., Bohm, M., Hasenfuss, G., Just, H., Holtz, J. and Drexler, H. (1994) Gene expression of the cardiac Na–Ca exchanger in end-stage human heart failure. Circ. Res. **75**, 443–453

80 Menick, D. R., Renaud, L., Buchholz, A., Muller, J. G., Zhou, H., Kappler, C. S., Kubalak, S. W., Conway, S. J. and Xu, L. (2007) Regulation of *Ncx1* gene expression in the normal and hypertrophic heart. Ann. N.Y. Acad. Sci. **1099**, 195–203

81 Xu, L., Renaud, L., Muller, J. G., Baicu, C. F., Bonnema, D. D., Zhou, H., Kappler, C. S., Kubalak, S. W., Zile, M. R., Conway, S. J. and Menick, D. R. (2006) Regulation of *Ncx1* expression: identification of regulatory elements mediating cardiac-specific expression and up-regulation. J. Biol. Chem. **281**, 34430–34440

82 Li, L., Guerini, D. and Carafoli, E. (2000) Calcineurin controls the transcription of Na^+/Ca^{2+} exchanger isoforms in developing cerebellar neurons. J. Biol. Chem. **275**, 20903–20910

83 Gomez-Villafuertes, R., Torres, B., Barrio, J., Savignac, M., Gabellini, N., Rizzato, F., Pintado, B., Gutierrez-Adan, A., Mellstrom, B., Carafoli, E. and Naranjo, J. R. (2005) Downstream regulatory element antagonist modulator regulates Ca^{2+} homeostasis and viability in cerebellar neurons. J. Neurosci. **25**, 10822–10830

84 Blaustein, M. P., DiPolo, R. and Reeves, J. P. (eds) (1991) Sodium–Calcium Exchange: Proceedings of the Second International Conference. Ann. N.Y. Acad. Sci. **639**, 1–671

85 Matsuoka, S. and Hilgemann, D. W. (1992) Steady-state and dynamic properties of cardiac sodium–calcium exchange: ion and voltage dependencies of the transport cycle. J. Gen. Physiol. **100**, 963–1001

86 DiPolo, R. and Beauge, L. (2006) Sodium/calcium exchanger: influence of metabolic regulation on ion carrier interactions. Physiol. Rev. **86**, 155–203

87 DiPolo, R. (1979) Calcium influx in internally dialyzed squid giant axons. J. Gen. Physiol. **73**, 91–113

88 Hilgemann, D. W. (1989) Giant excised cardiac sarcolemmal membrane patches: sodium and sodium–calcium exchange currents. Pflügers Arch. **415**, 247–249

89 Hilgemann, D. W. (1990) Regulation and deregulation of cardiac Na^+–Ca^{2+} exchange in giant excised sarcolemmal membrane patches. Nature **344**, 242–245

90 Chernysh, O., Condrescu, M. and Reeves, J. P. (2004) Calcium-dependent regulation of calcium efflux by the cardiac sodium/calcium exchanger. Am. J. Physiol. Cell Physiol. **287**, C797–C806

91 Ottolia, M., Philipson, K. D. and John, S. (2004) Conformational changes of the Ca^{2+} regulatory site of the Na^+–Ca^{2+} exchanger detected by FRET. Biophys. J. **87**, 899–906

92 Levitsky, D. O., Nicoll, D. A. and Philipson, K. D. (1994) Identification of the high affinity Ca-binding domain of the cardiac Na–Ca exchanger. J. Biol. Chem. **269**, 22847–22852

93 Matsuoka, S., Nicoll, D. A., Hryshko, L. V., Levitsky, D. O., Weiss, J. N. and Philipson, K. D. (1995) Regulation of the cardiac Na^+–Ca^{2+} exchanger by Ca^{2+}: mutational analysis of the Ca^{2+}-binding domain. J. Gen. Physiol. **105**, 403–420

94 Hryshko, L. V., Matsuoka, S., Nicoll, D. A., Weiss, J. N., Schwarz, E. M., Benzer, S. and Philipson, K. D. (1996) Anomalous regulation of the *Drosophila* Na^+–Ca^{2+} exchanger by Ca^{2+}. J. Gen. Physiol. **108**, 67–74

95 Dyck, C., Maxwell, K., Buchko, J., Trac, M., Omelchenko, A., Hnatowich, M. and Hryshko, L. V. (1998) Structure–function analysis of CALX1.1, a Na^+-Ca^{2+} exchanger from *Drosophila*: mutagenesis of ionic regulatory sites. J. Biol. Chem. **273**, 12981–12987

96 Hilgemann, D. W., Matsuoka, S., Nagel, G. A. and Collins, A. (1992) Steady-state and dynamic properties of cardiac sodium–calcium exchange: sodium-dependent inactivation. J. Gen. Physiol. **100**, 905–932

97 Matsuoka, S., Nicoll, D. A., He, Z. and Philipson, K. D. (1997) Regulation of cardiac Na^+–Ca^{2+} exchanger by the endogenous XIP region. J. Gen. Physiol. **109**, 273–286

98 Hilgemann, D. W., Collins, A. and Matsuoka, S. (1992) Steady-state and dynamic properties of cardiac sodium–calcium exchange: secondary modulation by cytoplasmic calcium and ATP. J. Gen. Physiol. **100**, 933–961

99 Hilgemann, D. W. and Ball, R. (1996) Regulation of cardiac Na^+,Ca^{2+} exchange and KATP potassium channels by PIP_2. Science **273**, 956–959

100 He, Z., Feng, S., Tong, Q., Hilgemann, D. W. and Philipson, K. D. (2000) Interaction of PIP_2 with the XIP region of the cardiac Na/Ca exchanger. Am. J. Physiol. Cell Physiol. **278**, C661–C666

101 Ander, B. P., Hurtado, C., Raposo, C. S., Maddaford, T. G., Deniset, J. F., Hryshko, L. V., Pierce, G. N. and Lukas, A. (2007) Differential sensitivities of the NCX1.1 and NCX1.3 isoforms of the Na^+–Ca^{2+} exchanger to α-linolenic acid. Cardiovasc. Res. **73**, 395–403

102 Condrescu, M. and Reeves, J. P. (2001) Inhibition of sodium–calcium exchange by ceramide and sphingosine. J. Biol. Chem. **276**, 4046–4054

103 Vemuri, R. and Philipson, K. D. (1988) Phospholipid composition modulates the Na^+-Ca^{2+} exchange activity of cardiac sarcolemma in reconstituted vesicles. Biochim. Biophys. Acta **937**, 258–268

104 Xiao, Y. F., Ke, Q., Chen, Y., Morgan, J. P. and Leaf, A. (2004) Inhibitory effect of *n*−3 fish oil fatty acids on cardiac Na^+/Ca^{2+} exchange currents in HEK293t cells. Biochem. Biophys. Res. Commun. **321**, 116–123

105 Riedel, M. J., Baczko, I., Searle, G. J., Webster, N., Fercho, M., Jones, L., Lang, J., Lytton, J., Dyck, J. R. and Light, P. E. (2006) Metabolic regulation of sodium–calcium exchange by intracellular acyl CoAs. EMBO J. **25**, 4605–4614

106 Doering, A. E. and Lederer, W. J. (1994) The action of Na^+ as a cofactor in the inhibition by cytoplasmic protons of the cardiac Na^+–Ca^{2+} exchanger in the guinea-pig. J. Physiol. **480**, 9–20

107 Dyck, C., Omelchenko, A., Elias, C. L., Quednau, B. D., Philipson, K. D., Hnatowich, M. and Hryshko, L. V. (1999) Ionic regulatory properties of brain and kidney splice variants of the NCX1 Na^+–Ca^{2+} exchanger. J. Gen. Physiol. **114**, 701–711

108 Dunn, J., Elias, C. L., Le, H. D., Omelchenko, A., Hryshko, L. V. and Lytton, J. (2002) The molecular determinants of ionic regulatory differences between brain and kidney Na^+/Ca^{2+} exchanger (NCX1) isoforms. J. Biol. Chem. **277**, 33957–33962

109 Reeves, J. P., Condrescu, M., Urbanczyk, J. and Chernysh, O. (2007) New modes of exchanger regulation: physiological implications. Ann. N.Y. Acad. Sci. **1099**, 64–77

110 Frank, J. S., Mottino, G., Reid, D., Molday, R. S. and Philipson, K. D. (1992) Distribution of the Na–Ca exchange protein in mammalian cardiac myocytes: an immunofluorescence and immunocolloidal gold-labeling study. J. Cell Biol. **117**, 337–345

111 Moore, E. D., Etter, E. F., Philipson, K. D., Carrington, W. A., Fogarty, K. E., Lifshitz, L. M. and Fay, F. S. (1993) Coupling of the Na^+/Ca^{2+} exchanger, Na^+/K^+ pump and sarcoplasmic reticulum in smooth muscle. Nature **365**, 657–660

112 Reilly, R. F., Shugrue, C. A., Lattanzi, D. and Biemesderfer, D. (1993) Immunolocalization of the Na^+/Ca^{2+} exchanger in rabbit kidney. Am. J. Physiol. Renal Physiol. **265**, F327–F332

113 Li, Z., Burke, E. P., Frank, J. S., Bennett, V. and Philipson, K. D. (1993) The cardiac Na^+–Ca^{2+} exchanger binds to the cytoskeletal protein ankyrin. J. Biol. Chem. **268**, 11489–11491

114 Mohler, P. J., Davis, J. Q. and Bennett, V. (2005) Ankyrin-B coordinates the Na/K ATPase, Na/Ca exchanger, and InsP_3 receptor in a cardiac T-tubule/SR microdomain. PLoS Biol. **3**, e423

115 Cunha, S. R., Bhasin, N. and Mohler, P. J. (2007) Targeting and stability of Na/Ca exchanger 1 in cardiomyocytes requires direct interaction with the membrane adaptor ankyrin-B. J. Biol. Chem. **282**, 4875–4883

116 Condrescu, M., Gardner, J. P., Chernaya, G., Aceto, J. F., Kroupis, C. and Reeves, J. P. (1995) ATP-dependent regulation of sodium–calcium exchange in Chinese hamster ovary cells transfected with the bovine cardiac sodium–calcium exchanger. J. Biol. Chem. **270**, 9137–9146

117 Condrescu, M. and Reeves, J. P. (2006) Actin-dependent regulation of the cardiac Na^+/Ca^{2+} exchanger. Am. J. Physiol. Cell Physiol. **290**, C691–C701

118 Razani, B., Woodman, S. E. and Lisanti, M. P. (2002) Caveolae: from cell biology to animal physiology. Pharmacol. Rev. **54**, 431–467

119 Williams, T. M. and Lisanti, M. P. (2004) The caveolin genes: from cell biology to medicine. Ann. Med. **36**, 584–595

120 Isshiki, M. and Anderson, R. G. (2003) Function of caveolae in Ca^{2+} entry and Ca^{2+}-dependent signal transduction. Traffic **4**, 717–723

121 Gratton, J. P., Bernatchez, P. and Sessa, W. C. (2004) Caveolae and caveolins in the cardiovascular system. Circ. Res. **94**, 1408–1417

122 Woodman, S. E., Park, D. S., Cohen, A. W., Cheung, M. W., Chandra, M., Shirani, J., Tang, B., Jelicks, L. A., Kitsis, R. N., Christ, G. J. et al. (2002) Caveolin-3 knock-out mice develop a progressive cardiomyopathy and show hyperactivation of the p42/44 MAPK cascade. J. Biol. Chem. **277**, 38988–38997

123 Scriven, D. R., Klimek, A., Asghari, P., Bellve, K. and Moore, E. D. (2005) Caveolin-3 is adjacent to a group of extradyadic ryanodine receptors. Biophys. J. **89**, 1893–1901

124 Bossuyt, J., Taylor, B. E., James-Kracke, M. and Hale, C. C. (2002) Evidence for cardiac sodium–calcium exchanger association with caveolin-3. FEBS Lett. **511**, 113–117

125 Camors, E., Charue, D., Trouve, P., Monceau, V., Loyer, X., Russo-Marie, F. and Charlemagne, D. (2006) Association of annexin A5 with Na^+/Ca^{2+} exchanger and caveolin-3 in non-failing and failing human heart. J. Mol. Cell. Cardiol. **40**, 47–55

126 Cavalli, A., Eghbali, M., Minosyan, T. Y., Stefani, E. and Philipson, K. D. (2007) Localization of sarcolemmal proteins to lipid rafts in the myocardium, Cell Calcium **42**, 313–322

127 Cha, S. H., Shin, S. Y., Jung, S. Y., Kim, Y. T., Park, Y. J., Kwak, J. O., Kim, H. W. and Suh, C. K. (2004) Evidence for Na^+/Ca^{2+} exchanger 1 association with caveolin-1 and -2 in C6 glioma cells. IUBMB Life **56**, 621–627

128 Cheung, J. Y., Rothblum, L. I., Moorman, J. R., Tucker, A. L., Song, J., Ahlers, B. A., Carl, L. L., Wang, J. and Zhang, X. Q. (2007) Regulation of cardiac Na^+/Ca^{2+} exchanger by phospholemman. Ann. N.Y. Acad. Sci. **1099**, 119–134

129 Garty, H. and Karlish, S. J. (2006) Role of FXYD proteins in ion transport. Annu. Rev. Physiol. **68**, 431–459

130 Song, J., Zhang, X. Q., Ahlers, B. A., Carl, L. L., Wang, J., Rothblum, L. I., Stahl, R. C., Mounsey, J. P., Tucker, A. L., Moorman, J. R. and Cheung, J. Y. (2005) Serine 68 of phospholemman is critical in modulation of contractility, $[Ca^{2+}]_i$ transients, and Na^+/Ca^{2+} exchange in adult rat cardiac myocytes. Am. J. Physiol. Heart Circ. Physiol. **288**, H2342–H2354

131 Wang, J., Zhang, X. Q., Ahlers, B. A., Carl, L. L., Song, J., Rothblum, L. I., Stahl, R. C., Carey, D. J. and Cheung, J. Y. (2006) Cytoplasmic tail of phospholemman interacts with the intracellular loop of the cardiac Na^+/Ca^{2+} exchanger. J. Biol. Chem. **281**, 32004–32014

132 Zhang, X. Q., Ahlers, B. A., Tucker, A. L., Song, J., Wang, J., Moorman, J. R., Mounsey, J. P., Carl, L. L., Rothblum, L. I. and Cheung, J. Y. (2006) Phospholemman inhibition of the cardiac Na^+/Ca^{2+} exchanger: role of phosphorylation. J. Biol. Chem. **281**, 7784–7792

133 Tucker, A. L., Song, J., Zhang, X. Q., Wang, J., Ahlers, B. A., Carl, L. L., Mounsey, J. P., Moorman, J. R., Rothblum, L. I. and Cheung, J. Y. (2006) Altered contractility and $[Ca^{2+}]_i$ homeostasis in phospholemman-deficient murine myocytes: role of Na^+/Ca^{2+} exchange. Am. J. Physiol. Heart Circ. Physiol. **291**, H2199–H2209

134 Crambert, G., Fuzesi, M., Garty, H., Karlish, S. and Geering, K. (2002) Phospholemman (FXYD1) associates with Na,K-ATPase and regulates its transport properties. Proc. Natl. Acad. Sci. U.S.A. **99**, 11476–11481

135 Katanosaka, Y., Iwata, Y., Kobayashi, Y., Shibasaki, F., Wakabayashi, S. and Shigekawa, M. (2005) Calcineurin inhibits Na^+/Ca^{2+} exchange in phenylephrine-treated hypertrophic cardiomyocytes. J. Biol. Chem. **280**, 5764–5772

136 Pulina, M. V., Rizzuto, R., Brini, M. and Carafoli, E. (2006) Inhibitory interaction of the plasma membrane Na^+/Ca^{2+} exchangers with the 14-3-3 proteins. J. Biol. Chem. **281**, 19645–19654

137 Bridges, D. and Moorhead, G. B. (2005) 14-3-3 proteins: a number of functions for a numbered protein. Science STKE **2005**, re10

138 Zhang, Y. H., Hinde, A. K. and Hancox, J. C. (2001) Anti-adrenergic effect of adenosine on Na^+–Ca^{2+} exchange current recorded from guinea-pig ventricular myocytes. Cell Calcium **29**, 347–358

139 Perchenet, L., Hinde, A. K., Patel, K. C., Hancox, J. C. and Levi, A. J. (2000) Stimulation of Na/Ca exchange by the β-adrenergic/protein kinase A pathway in guinea-pig ventricular myocytes at 37 °C. Pflügers Arch. **439**, 822–828

140 Wei, S. K., Ruknudin, A., Hanlon, S. U., McCurley, J. M., Schulze, D. H. and Haigney, M. C. (2003) Protein kinase A hyperphosphorylation increases basal current but decreases β-adrenergic responsiveness of the sarcolemmal Na^+–Ca^{2+} exchanger in failing pig myocytes. Circ. Res. **92**, 897–903

141 Ruknudin, A., He, S., Lederer, W. J. and Schulze, D. H. (2000) Functional differences between cardiac and renal isoforms of the rat Na^+–Ca^{2+} exchanger NCX1 expressed in *Xenopus* oocytes. J. Physiol. **529**, 599–610

142 Ginsburg, K. S. and Bers, D. M. (2005) Isoproterenol does not enhance Ca-dependent Na/Ca exchange current in intact rabbit ventricular myocytes. J. Mol. Cell. Cardiol. **39**, 972–981

143 Lin, X., Jo, H., Sakakibara, Y., Tambara, K., Kim, B., Komeda, M. and Matsuoka, S. (2006) β-Adrenergic stimulation does not activate Na^+/Ca^{2+} exchange current in guinea pig, mouse, and rat ventricular myocytes. Am. J. Physiol. Cell Physiol. **290**, C601–C608

144 Haigney, M. C., Wei, S. K., Schulze, D. H., Ruknudin, A. M. and Matsuoka, S. (2006) Response to "β-Adrenergic stimulation does not activate Na^+/Ca^{2+} exchange current in guinea pig, mouse, and rat ventricular myocytes". Am. J. Physiol. Cell Physiol. **290**, C1271

145 Iwamoto, T., Pan, Y., Wakabayashi, S., Imagawa, T., Yamanaka, H. I. and Shigekawa, M. (1996) Phosphorylation-dependent regulation of cardiac Na^+/Ca^{2+} exchanger via protein kinase C. J. Biol. Chem. **271**, 13609–13615

146 Iwamoto, T., Pan, Y., Nakamura, T. Y., Wakabayashi, S. and Shigekawa, M. (1998) Protein kinase C-dependent regulation of Na^+/Ca^{2+} exchanger isoforms NCX1 and NCX3 does not require their direct phosphorylation. Biochemistry **37**, 17230–17238

147 Iwamoto, T. (2007) Na^+/Ca^{2+} exchange as a drug target: insights from molecular pharmacology and genetic engineering. Ann. N.Y. Acad. Sci. **1099**, 516–528

148 Cho, C. H., Kim, S. S., Jeong, M. J., Lee, C. O. and Shin, H. S. (2000) The Na^+–Ca^{2+} exchanger is essential for embryonic heart development in mice. Mol. Cells **10**, 712–722

149 Wakimoto, K., Kobayashi, K., Kuro, O. M., Yao, A., Iwamoto, T., Yanaka, N., Kita, S., Nishida, A., Azuma, S., Toyoda, Y. et al. (2000) Targeted disruption of Na^+/Ca^{2+} exchanger gene leads to cardiomyocyte apoptosis and defects in heartbeat. J. Biol. Chem. **275**, 36991–36998

150 Koushik, S. V., Wang, J., Rogers, R., Moskophidis, D., Lambert, N. A., Creazzo, T. L. and Conway, S. J. (2001) Targeted inactivation of the sodium–calcium exchanger (Ncx1) results in the lack of a heartbeat and abnormal myofibrillar organization. FASEB J. **15**, 1209–1211

151 Reuter, H., Henderson, S. A., Han, T., Ross, R. S., Goldhaber, J. I. and Philipson, K. D. (2002) The Na^+–Ca^{2+} exchanger is essential for the action of cardiac glycosides. Circ. Res. **90**, 305–308

152 Cho, C. H., Lee, S. Y., Shin, H. S., Philipson, K. D. and Lee, C. O. (2003) Partial rescue of the Na^+–Ca^{2+} exchanger (NCX1) knock-out mouse by transgenic expression of NCX1. Exp. Mol. Med. **35**, 125–135

153 Henderson, S. A., Goldhaber, J. I., So, J. M., Han, T., Motter, C., Ngo, A., Chantawansri, C., Ritter, M. R., Friedlander, M., Nicoll, D. A. et al. (2004) Functional adult myocardium in the absence of Na^+–Ca^{2+} exchange: cardiac-specific knockout of NCX1. Circ. Res. **95**, 604–611

154 Pott, C., Yip, M., Goldhaber, J. I. and Philipson, K. D. (2007) Regulation of cardiac L-type Ca^{2+} current in Na^+–Ca^{2+} exchanger knockout mice: functional coupling of the Ca^{2+} channel and the Na^+–Ca^{2+} exchanger. Biophys. J. **92**, 1431–1437

155 Pott, C., Ren, X., Tran, D. X., Yang, M. J., Henderson, S., Jordan, M. C., Roos, K. P., Garfinkel, A., Philipson, K. D. and Goldhaber, J. I. (2007) Mechanism of shortened action potential duration in Na^+–Ca^{2+} exchanger knockout mice. Am. J. Physiol. Cell Physiol. **292**, C968–C973

156 Reuter, H., Pott, C., Goldhaber, J. I., Henderson, S. A., Philipson, K. D. and Schwinger, R. H. (2005) Na^+–Ca^{2+} exchange in the regulation of cardiac excitation–contraction coupling. Cardiovasc. Res. **67**, 198–207

157 Neco, P., Lamp, S. T., Bridge, J. H., Philipson, K. D. and Goldhaber, J. I. (2007) Na^+–Ca^{2+} exchange promotes normal triggering of Ca^{2+} release in mouse heart. Biophys. J. **92**, 624–628

158 Reuter, H. and Porzig, H. (1995) Localization and functional significance of the Na/Ca exchanger in presynaptic boutons of hippocampal cells in culture. Neuron **15**, 1077–1084

159 Minelli, A., Castaldo, P., Gobbi, P., Salucci, S., Magi, S. and Amoroso, S. (2007) Cellular and subcellular localization of Na^+–Ca^{2+} exchanger protein isoforms, NCX1, NCX2, and NCX3 in cerebral cortex and hippocampus of adult rat. Cell Calcium **41**, 221–234

160 Bano, D., Young, K. W., Guerin, C. J., Lefeuvre, R., Rothwell, N. J., Naldini, L., Rizzuto, R., Carafoli, E. and Nicotera, P. (2005) Cleavage of the plasma membrane Na^+/Ca^{2+} exchanger in excitotoxicity. Cell **120**, 275–285

161 Lorincz, A., Rozsa, B., Katona, G., Vizi, E. S. and Tamas, G. (2007) Differential distribution of NCX1 contributes to spine-dendrite compartmentalization in CA1 pyramidal cells. Proc. Natl. Acad. Sci. U.S.A. **104**, 1033–1038

162 Burdakov, D., Liss, B. and Ashcroft, F. M. (2003) Orexin excites GABAergic neurons of the arcuate nucleus by activating the sodium–calcium exchanger. J. Neurosci. **23**, 4951–4957

163 Eriksson, K. S., Sergeeva, O. A., Stevens, D. R. and Haas, H. L. (2002) Neurotransmitter-induced activation of sodium–calcium exchange causes neuronal excitation. Ann. N.Y. Acad. Sci. **976**, 405–407

164 Eriksson, K. S., Stevens, D. R. and Haas, H. L. (2001) Serotonin excites tuberomammillary neurons by activation of Na^+/Ca^{2+}-exchange. Neuropharmacology **40**, 345–351

165 Hoyt, K. R., Arden, S. R., Aizenman, E. and Reynolds, I. J. (1998) Reverse Na^+/Ca^{2+} exchange contributes to glutamate-induced intracellular Ca^{2+} concentration increases in cultured rat forebrain neurons. Mol. Pharmacol. **53**, 742–749

166 Sergeeva, O. A., Amberger, B. T., Vorobjev, V. S., Eriksson, K. S. and Haas, H. L. (2004) AMPA receptor properties and coexpression with sodium–calcium exchangers in rat hypothalamic neurons. Eur. J. Neurosci. **19**, 957–965

167 Nakasaki, Y., Iwamoto, T., Hanada, H., Imagawa, T. and Shigekawa, M. (1993) Cloning of the rat aortic smooth muscle Na^+/Ca^{2+} exchanger and tissue-specific expression of isoforms. J. Biochem. (Tokyo) **114**, 528–534

168 Dong, H., Jiang, Y., Triggle, C. R., Li, X. and Lytton, J. (2006) Novel role for K^+-dependent Na^+/Ca^{2+} exchangers in regulation of cytoplasmic free Ca^{2+} and contractility in arterial smooth muscle. Am. J. Physiol. Heart Circ. Physiol. **291**, H1226–H1235

169 Hirota, S., Pertens, E. and Janssen, L. J. (2007) The reverse mode of the Na^+/Ca^{2+} exchanger provides a source of Ca^{2+} for store refilling following agonist-induced Ca^{2+} mobilization. Am. J. Physiol. Lung Cell. Mol. Physiol. **292**, L438–L447

170 Lemos, V. S., Poburko, D., Liao, C. H., Cole, W. C. and van Breemen, C. (2007) Na^+ entry via TRPC6 causes Ca^{2+} entry via NCX reversal in ATP stimulated smooth muscle cells. Biochem. Biophys. Res. Commun. **352**, 130–134

171 Hoenderop, J. G., Nilius, B. and Bindels, R. J. (2005) Calcium absorption across epithelia. Physiol. Rev. **85**, 373–422

172 Augustine, G. J., Santamaria, F. and Tanaka, K. (2003) Local calcium signaling in neurons. Neuron **40**, 331–346

173 Malenka, R. C. and Bear, M. F. (2004) LTP and LTD: an embarrassment of riches. Neuron **44**, 5–21

174 Sokolow, S., Manto, M., Gailly, P., Molgo, J., Vandebrouck, C., Vanderwinden, J. M., Herchuelz, A. and Schurmans, S. (2004) Impaired neuromuscular transmission and skeletal muscle fiber necrosis in mice lacking Na/Ca exchanger 3. J. Clin. Invest. **113**, 265–273

175 Sipido, K. R., Bito, V., Antoons, G., Volders, P. G. and Vos, M. A. (2007) Na/Ca exchange and cardiac ventricular arrhythmias. Ann. N.Y. Acad. Sci. **1099**, 339–348

176 Venetucci, L. A., Trafford, A. W., O'Neill, S. C. and Eisner, D. A. (2007) Na/Ca exchange: regulator of intracellular calcium and source of arrhythmias in the heart. Ann. N.Y. Acad. Sci. **1099**, 315–325

177 Venetucci, L. A., Trafford, A. W. and Eisner, D. A. (2007) Increasing ryanodine receptor open probability alone does not produce arrhythmogenic calcium waves: threshold sarcoplasmic reticulum calcium content is required. Circ. Res. **100**, 105–111

178 Sipido, K. R., Volders, P. G., Vos, M. A. and Verdonck, F. (2002) Altered Na/Ca exchange activity in cardiac hypertrophy and heart failure: a new target for therapy? Cardiovasc. Res. **53**, 782–805

179 Sipido, K. R. (2006) Calcium overload, spontaneous calcium release, and ventricular arrhythmias. Heart Rhythm **3**, 977–979

180 Hobai, I. A., Maack, C. and O'Rourke, B. (2004) Partial inhibition of sodium/calcium exchange restores cellular calcium handling in canine heart failure. Circ. Res. **95**, 292–299

181 Murphy, E., Cross, H. and Steenbergen, C. (1999) Sodium regulation during ischemia versus reperfusion and its role in injury. Circ. Res. **84**, 1469–1470

182 Lipton, P. (1999) Ischemic cell death in brain neurons. Physiol. Rev. **79**, 1431–1568

183 Stys, P. K. (2005) General mechanisms of axonal damage and its prevention. J. Neurol. Sci. **233**, 3–13

184 Kiedrowski, L. (1999) *N*-methyl-D-aspartate excitotoxicity: relationships among plasma membrane potential, Na^+/Ca^{2+} exchange, mitochondrial Ca^{2+} overload, and cytoplasmic concentrations of Ca^{2+}, H^+, and K^+. Mol. Pharmacol. **56**, 619–632

185 Li, S., Jiang, Q. and Stys, P. K. (2000) Important role of reverse Na^+–Ca^{2+} exchange in spinal cord white matter injury at physiological temperature. J. Neurophysiol. **84**, 1116–1119

186 Kiedrowski, L. (2007) NCX and NCKX operation in ischemic neurons. Ann. N.Y. Acad. Sci. **1099**, 383–395

187 Pignataro, G., Gala, R., Cuomo, O., Tortiglione, A., Giaccio, L., Castaldo, P., Sirabella, R., Matrone, C., Canitano, A., Amoroso, S. et al. (2004) Two sodium/calcium exchanger gene products, NCX1 and NCX3, play a major role in the development of permanent focal cerebral ischemia. Stroke **35**, 2566–2570

188 Pignataro, G., Tortiglione, A., Scorziello, A., Giaccio, L., Secondo, A., Severino, B., Santagada, V., Caliendo, G., Amoroso, S., Di Renzo, G. and Annunziato, L. (2004) Evidence for a protective role played by the Na^+/Ca^{2+} exchanger in cerebral ischemia induced by middle cerebral artery occlusion in male rats. Neuropharmacology **46**, 439–448

189 Annunziato, L., Pignataro, G., Boscia, F., Sirabella, R., Formisano, L., Saggese, M., Cuomo, O., Gala, R., Secondo, A., Viggiano, D. et al. (2007) *ncx1*, *ncx2*, and *ncx3* gene product expression and function in neuronal anoxia and brain ischemia. Ann. N.Y. Acad. Sci. **1099**, 413–426

190 Iwamoto, T., Kita, S., Zhang, J., Blaustein, M. P., Arai, Y., Yoshida, S., Wakimoto, K., Komuro, I. and Katsuragi, T. (2004) Salt-sensitive hypertension is triggered by Ca^{2+} entry via Na^+/Ca^{2+} exchanger type-1 in vascular smooth muscle. Nat. Med. **10**, 1193–1199

191 Lee, C., Dhalla, N. S. and Hryshko, L. V. (2005) Therapeutic potential of novel Na^+–Ca^{2+} exchange inhibitors in attenuating ischemia-reperfusion injury. Can. J. Cardiol. **21**, 509–516

192 Noble, D. and Blaustein, M. P. (2007) Directionality in drug action on sodium–calcium exchange. Ann. N.Y. Acad. Sci. **1099**, 540–543

193 Schnetkamp, P. P. M. (1995) Calcium homeostasis in vertebrate retinal rod outer segments. Cell Calcium **18**, 322–330

194 Kimura, M., Jeanclos, E. M., Donnelly, R. J., Lytton, J. and Reeves, J. P. (1999) Physiological and molecular characterization of the Na^+/Ca^{2+} exchanger in human platelets. Am. J. Physiol. Heart Physiol. **277**, H911–H917

195 Lytton, J., Li, X. F., Dong, H. and Kraev, A. (2002) K^+-dependent Na^+/Ca^{2+} exchangers in the brain. Ann. N.Y. Acad. Sci. **976**, 382–393

196 Poon, S., Leach, S., Li, X. F., Tucker, J. E., Schnetkamp, P. P. M. and Lytton, J. (2000) Alternatively spliced isoforms of the rat eye sodium/calcium+potassium exchanger NCKX1. Am. J. Physiol. Cell Physiol. **278**, C651–C660

197 Prinsen, C. F., Szerencsei, R. T. and Schnetkamp, P. P. M. (2000) Molecular cloning and functional expression of the potassium-dependent sodium–calcium exchanger from human and chicken retinal cone photoreceptors. J. Neurosci. **20**, 1424–1434

198 Paillart, C., Winkfein, R. J., Schnetkamp, P. P. and Korenbrot, J. I. (2007) Functional characterization and molecular cloning of the K^+-dependent Na^+/Ca^{2+} exchanger in intact retinal cone photoreceptors. J. Gen. Physiol. **129**, 1–16

199 Sharon, D., Yamamoto, H., McGee, T. L., Rabe, V., Szerencsei, R. T., Winkfein, R. J., Prinsen, C. F., Barnes, C. S., Andreasson, S., Fishman, G. A. et al. (2002) Mutated alleles of the rod and cone Na–Ca+K-exchanger genes in patients with retinal diseases. Invest. Ophthalmol. Visual Sci. **43**, 1971–1979

200 Kang, K. and Schnetkamp, P. P. (2003) Signal sequence cleavage and plasma membrane targeting of the retinal rod NCKX1 and cone NCKX2 Na^+/Ca^{2+}–K^+ exchangers. Biochemistry **42**, 9438–9445

201 Cai, X., Zhang, K. and Lytton, J. (2002) A novel topology and redox regulation of the rat brain K^+-dependent Na^+/Ca^{2+} exchanger, NCKX2. J. Biol. Chem. **277**, 48923–48930

202 Kang, K. J., Kinjo, T. G., Szerencsei, R. T. and Schnetkamp, P. P. (2005) Residues contributing to the Ca^{2+} and K^+ binding pocket of the NCKX2 Na^+/Ca^{2+}–K^+ exchanger. J. Biol. Chem. **280**, 6823–6833

203 Kang, K. J., Shibukawa, Y., Szerencsei, R. T. and Schnetkamp, P. P. (2005) Substitution of a single residue, Asp^{575}, renders the NCKX2 K^+-dependent Na^+/Ca^{2+} exchanger independent of K^+. J. Biol. Chem. **280**, 6834–6839

204 Visser, F., Valsecchi, V., Annunziato, L. and Lytton, J. (2007) Analysis of ion interactions with the K^+-dependent Na^+/Ca^{2+} Exchangers NCKX2, NCKX3, and NCKX4: identification of Thr-551 as a key residue in defining the apparent K^+ affinity of NCKX2. J. Biol. Chem. **282**, 4453–4462

205 Lee, J. Y., Ho, W. K. and Lee, S. H. (2007) Ionic selectivity of NCKX2, NCKX3, and NCKX4 for monovalent cations at K^+-binding site. Ann. N.Y. Acad. Sci. **1099**, 166–170

206 Schnetkamp, P. P. (2004) The SLC24 Na^+/Ca^{2+}–K^+ exchanger family: vision and beyond. Pflügers Arch. **447**, 683–688

207 Kaupp, U. B. and Seifert, R. (2002) Cyclic nucleotide-gated ion channels. Physiol. Rev. **82**, 769–824

208 Schwarzer, A., Schauf, H. and Bauer, P. J. (2000) Binding of the cGMP-gated channel to the Na/Ca–K exchanger in rod photoreceptors. J. Biol. Chem. **275**, 13448–13454

209 Kang, K., Bauer, P. J., Kinjo, T. G., Szerencsei, R. T., Bonigk, W., Winkfein, R. J. and Schnetkamp, P. P. (2003) Assembly of retinal rod or cone Na^+/Ca^{2+}–K^+ exchanger oligomers with cGMP-gated channel subunits as probed with heterologously expressed cDNAs. Biochemistry **42**, 4593–4600

210 Yoo, S. S., Leach, S. and Lytton, J. (2002) Studies on the oligomeric state of the sodium/calcium+potassium exchanger NCKX2. Ann. N.Y. Acad. Sci. **976**, 94–96

211 Altimimi, H. F. and Schnetkamp, P. P. (2007) Na^+-dependent inactivation of the retinal cone/brain Na^+/Ca^{2+}–K^+ exchanger NCKX2. J. Biol. Chem. **282**, 3720–3729

212 Lee, J. Y., Visser, F., Lee, J. S., Lee, K. H., Soh, J. W., Ho, W. K., Lytton, J. and Lee, S. H. (2006) Protein kinase C-dependent enhancement of activity of rat brain NCKX2 heterologously expressed in HEK293 cells. J. Biol. Chem. **281**, 39205–39216

213 Lan, J. Y., Skeberdis, V. A., Jover, T., Grooms, S. Y., Lin, Y., Araneda, R. C., Zheng, X., Bennett, M. V. and Zukin, R. S. (2001) Protein kinase C modulates NMDA receptor trafficking and gating. Nat. Neurosci. **4**, 382–390

214 Lou, X., Scheuss, V. and Schneggenburger, R. (2005) Allosteric modulation of the presynaptic Ca^{2+} sensor for vesicle fusion. Nature **435**, 497–501

215 Fain, G. L., Matthews, H. R., Cornwall, M. C. and Koutalos, Y. (2001) Adaptation in vertebrate photoreceptors. Physiol. Rev. **81**, 117–151

216 Kim, M. H., Lee, S. H., Park, K. H. and Ho, W. K. (2003) Distribution of K^+-dependent Na^+/Ca^{2+} exchangers in the rat supraoptic magnocellular neuron is polarized to axon terminals. J. Neurosci. **23**, 11673–11680

217 Kiedrowski, L. (2004) High activity of K^+-dependent plasmalemmal Na^+/Ca^{2+} exchangers in hippocampal CA1 neurons. NeuroReport **15**, 2113–2116

218 Chi, A., Valencia, J. C., Hu, Z. Z., Watabe, H., Yamaguchi, H., Mangini, N. J., Huang, H., Canfield, V. A., Cheng, K. C., Yang, F. et al. (2006) Proteomic and bioinformatic characterization of the biogenesis and function of melanosomes. J. Proteome Res. **5**, 3135–3144

219 Pyrski, M., Koo, J. H., Polumuri, S. K., Ruknudin, A. M., Margolis, J. W., Schulze, D. H. and Margolis, F. L. (2007) Sodium/calcium exchanger expression in the mouse and rat olfactory systems. J. Comp. Neurol. **501**, 944–958

220 Boyer, C., Sans, A., Vautrin, J., Chabbert, C. and Lehouelleur, J. (1999) K^+-dependence of Na^+–Ca^{2+} exchange in type I vestibular sensory cells of guinea-pig. Eur. J. Neurosci. **11**, 1955–1959

221 Su, Y. H. and Vacquier, V. D. (2002) A flagellar K^+-dependent Na^+/Ca^{2+} exchanger keeps Ca^{2+} low in sea urchin spermatozoa. Proc. Natl. Acad. Sci. U.S.A. **99**, 6743–6748

222 Hansen, A. J. and Zeuthen, T. (1981) Extracellular ion concentrations during spreading depression and ischemia in the rat brain cortex. Acta Physiol. Scand. **113**, 437–445
223 Kiedrowski, L., Czyz, A., Baranauskas, G., Li, X. F. and Lytton, J. (2004) Differential contribution of plasmalemmal Na/Ca exchange isoforms to sodium-dependent calcium influx and NMDA excitotoxicity in depolarized neurons. J. Neurochem. **90**, 117–128
224 Cuomo, O., Pignataro, G., Gala, R., Boscia, F., Tortiglione, A., Molinaro, P., Di Renzo, G., Lytton, J. and Annunziato, L. (2007) Involvement of the potassium-dependent sodium/calcium exchanger gene product NCKX2 in the brain insult induced by permanent focal cerebral ischemia. Ann. N.Y. Acad. Sci. **1099**, 486–489
225 Gabellini, N., Zatti, A., Rispoli, G., Navangione, A. and Carafoli, E. (1996) Expression of an active Na^+/Ca^{2+} exchanger isoform lacking the six C-terminal transmembrane segments. Eur. J. Biochem. **239**, 897–904
226 Li, X. F. and Lytton, J. (1999) A circularized sodium–calcium exchanger exon 2 transcript. J. Biol. Chem. **274**, 8153–8160
227 Palty, R., Hershfinkel, M., Yagev, O., Saar, D., Barkalifa, R., Khananshvili, D., Peretz, A., Grossman, Y. and Sekler, I. (2006) Single α-domain constructs of the Na^+/Ca^{2+} exchanger, NCLX, oligomerize to form a functional exchanger. Biochemistry **45**, 11856–11866
228 Saaf, A., Baars, L. and von Heijne, G. (2001) The internal repeats in the Na^+/Ca^{2+} exchanger-related *Escherichia coli* protein YrbG have opposite membrane topologies. J. Biol. Chem. **276**, 18905–18907
229 Herchuelz, A., Blaustein, M. P., Lytton, J. and Philipson, K. D. (eds) (2007) Sodium–Calcium Exchange and the Plasma Membrane Ca^{2+}-ATPase in Cell Function: Fifth International Conference. Ann. N.Y. Acad. Sci. **1099**, xv–547

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